โครงการ Phylogeography of seaweeds in Southeast Asia

โดย Dr. Stefano Draisma

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รายงานวิจัยฉบับสมบูรณ์

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3. **รูปแบบ Abstract (บทคัดย่อ)**

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เนื้อหางานวิจัยประกอบด้วย วัตถุประสงค์ วิธีทดลอง ผลการทดลอง สรุปและวิจารณ์ผลการทดลอง และข้อเสนอแนะสำหรับงานวิจัยในอนาคต

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The centre of marine biodiversity, a.k.a. the Coral Triangle, is located in Southeast Asia. Quarternary sea level fluctuations, present-day ocean currents, and organismal dispersal ability are believed to be responsible for the distribution of lineages. Barriers in the marine realm are not conspicuous and dispersal abilities of marine organisms are hard to determine, but surveys of the distribution of intraspecific genetic variation (phylogeography) provide an indirect means of detecting connectivities between populations. The present study aims to explore the phylogeography of two red algae in Southeast Asia, *i.e.*, circumtropical *Dichotomaria marginata* and Indo-Pacific *Gibsmithia hawaiiensis*. Based on variability and amplifyability, the chloroplast-encoded 3'-*rbc*L gene was chosen as genetic marker for *Dichotomaria* and the mitochondrial *cox*2,3 spacer for *Gib-smithia*. *Gibsmithia hawaiiensis* was only found in Taiwan and the Pacific coast of the Philip-pines, albeit genetically different from each other and from Hawaiian populations (type location), likely as a result of isolation-by-distance. *G. indopacifica* nov. sp. and *G. malayensis*. nov. sp. are widely distributed in, respectively, Southeast Asia and the Coral Triangle. These are cryptic species as they are not distinguishable from each other based on their macromorphology. Four more undescribed cryptic *Gibsmithia* species were found, each with a limited distribution. The Pacific Philippines appears to be the center of *Gibsmithia* diversity with five of seven Southeast Asian species. There appears to be low connectivity between populations of *G. indopacifica* and *G. malayensis* from the Sulu Sea, Halmahera Sea, and Molucca Sea. The type location of *Dichotomaria marginata* is in the Caribbean, but DNA sequence data indicate the occurrence of two Caribbean flattened *Dichotomaria* species, of which one is also widespread in Southeast Asia. An unknown flattened *Dichotomaria* species was found in Borneo. The *D. marginata rbc*L haplotype distribution pattern indicates that there is probably higher connectivity in this species (compared to *Gibsmithia* spp.), but the Halmahera Sea population also appears to be isolated, likely as a consequence of the Halmahera eddy. More specimens will be haplotyped to get a clearer picture of the phylogeographic patterns of these algae and the newly discovered species will be examined for diagnostic characters.

Keywords : จำนวน 3-5 คำ cryptic species, haplotype diversity, new species, phylogeography, red algae

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ศูนย์กลางความหลากหลายทางชีวภาพทางทะเล ซึ่งเป็นที่รู้จักกันในชื่อ Coral Triangle ตั้งอยู่ในภูมิภาคเอเชียตะวันออกเฉียงใต้ ความผันแปรของระดับน้ำทะเลในยุคปัจจุบัน (Quaternary) กระแสน้ำในมหาสมุทรและความสามารถในการแพร่กระจายของสิ่งมีชีวิตถูกเชื่อว่าเป็นมูลเหตุในการแพร่กระจายของสายพันธุ์ สิ่งกีดขวางทางทะเลไม่มีความชัดเจนและความสามารถในการแพร่กระจายของสิ่งมีชีวิตทางทะเลนั้นยากที่จะศึกษา แต่การสำรวจการแพร่กระจายของความแปรผันทางพันธุกรรม (phylogeography) เป็นวิธีการทางอ้อมในการตรวจสอบความเชื่อมโยงระหว่างประชากร การศึกษาครั้งนี้มีจุดมุ่งหมายเพื่อศึกษา phylogeography ของสาหร่ายสีแดงสองชนิดในเอเชียตะวันออกเฉียงใต้ ได้แก่ *Dichotomaria marginata* เขตร้อนและ*Gibsmithia hawaiiensis* อินโดแปซิฟิกโดยใช้ยีน rbcL ในคลอโรพลาสเป็นตัวบ่งชี้ทางพันธุกรรมของ *Dichotomaria*และยีน cox2,3 ในไมโทคอนเดียสำหรับ *Gibsmithia Gibsmithia hawaiiensis* ถูกพบเฉพาะในไต้หวันและชายฝั่งแปซิฟิกของฟิลิปส์ปินส์ แม้ว่าจะมีความแตกต่างทางพันธุกรรมกันและต่างจากประชากรฮาวาย (Type location)ราวกับว่าเป็นผลมาจากการแยกกันโดยระยะทาง *G. indopacifica* nov. sp. และ *G. malayensis* nov. sp. มีการแพร่กระจายอย่างกว้างขวางในเอเชียตะวันออกเฉียงใต้และ Coral Triangle ตามลำดับ ซึ่งสายพันธุ์เหล่านี้เป็นชนิดที่คลุมเครือที่ไม่สามารถแยกความแตกต่างของลักษณะทางสัณฐานวิทยาได้ และยังพบสายพันธุ์ *Gibsmithia* ที่ไม่สามารถระบุชนิดได้อีก 4 ชนิดโดยแต่ละชนิดมีการแพร่กระจายตัวในวงจำกัด ฝั่งมหาสมุทรแปซิฟิกของฟิลิปปินส์เสมือนเป็นศูนย์กลางของความหลากหลายของ *Gibsmithia* ซึ่งมี 5 ชนิดจาก 7 ชนิดที่พบในเอเชียตะวันออกเฉียงใต้ และเสมือนว่ามีความเชื่อมโยงต่ำระหว่างประชากรของ *G. indopacifica*และ *G. malayensis* จากทะเลซูลู ทะเลฮามาเฮอลาและทะเลมะลัคคา ซึ่ง Type location ที่พบ *Dichotomaria marginata* ตั้งอยู่ในทะเลแคริบเบียน แต่ข้อมูลลำดับดีเอ็นเอบ่งชี้ว่าพบ *Dichotomaria* 2ชนิดที่พบในแคริเบียนที่มีลักษณะแผ่นใบแบน หนึ่งชนิดในนั้นมีการแพร่กระจายไปยังเอเชียตะวันออกเฉียงใต้ อีกทั้งพบ *Dichotomaria* ซึ่งมีลักษณะแผ่นใบแบนและไม่ทราบชนิดในบอร์เนียว รูปแบบการแพร่กระจายhaplotype ของ *D. marginata* โดยใช้ยีน rbcL ระบุว่าอาจมีการเชื่อมโยงสูงกว่าในสายพันธุ์นี้ (เทียบกับ *Gibsmithia* spp.) แต่ประชากรในทะเลฮามาเฮอลาคล้ายว่าจะถูกแยกและไม่มีการเชื่อมโยง ซึ่งอาจเป็นผลมาจาก Halmahera Eddy หากมีตัวอย่างเพิ่มเติมมากขึ้นก็จะช่วยให้เห็นภาพที่ชัดเจนของรูปแบบ phylogeography ของสาหร่ายเหล่านี้และสายพันธุ์ที่เพิ่งค้นพบจะทำการศึกษาลักษณะสัณฐานต่อไป

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4. เนื้อหางานวิจัย (บทนำ วิธีการทดลอง ผลการทดลอง บทวิจารณ์ หนังสืออ้างอิง)

Introduction

The center of marine biodiversity, often referred to as the Coral Triangle (Briggs 2003, Gaither *et al.* 2011) is situated in the Indo-Pacific transition zone. Here the so called Indonesian through-flow squeezes ocean currents from the Pacific through the Indo-Malay archipelago into the Indian Ocean, transporting organismal propagules (*e.g.*, larvae, zygotes, spores, detached fragments) which have the potential to replenish downstream reefs. Geographical barriers in the marine realm are not conspicuous and dispersal abilities of marine organisms are hard to determine. However, an indirect means of tracing movements made between marine populations by propagules is by making geographical surveys of intraspecific genetic variation (Mirams *et al.* 2011). Repeated glacial cycles during the Pleistocene are believed to have caused isolation of marine taxa in refugia, resulting in diversification of lineages.

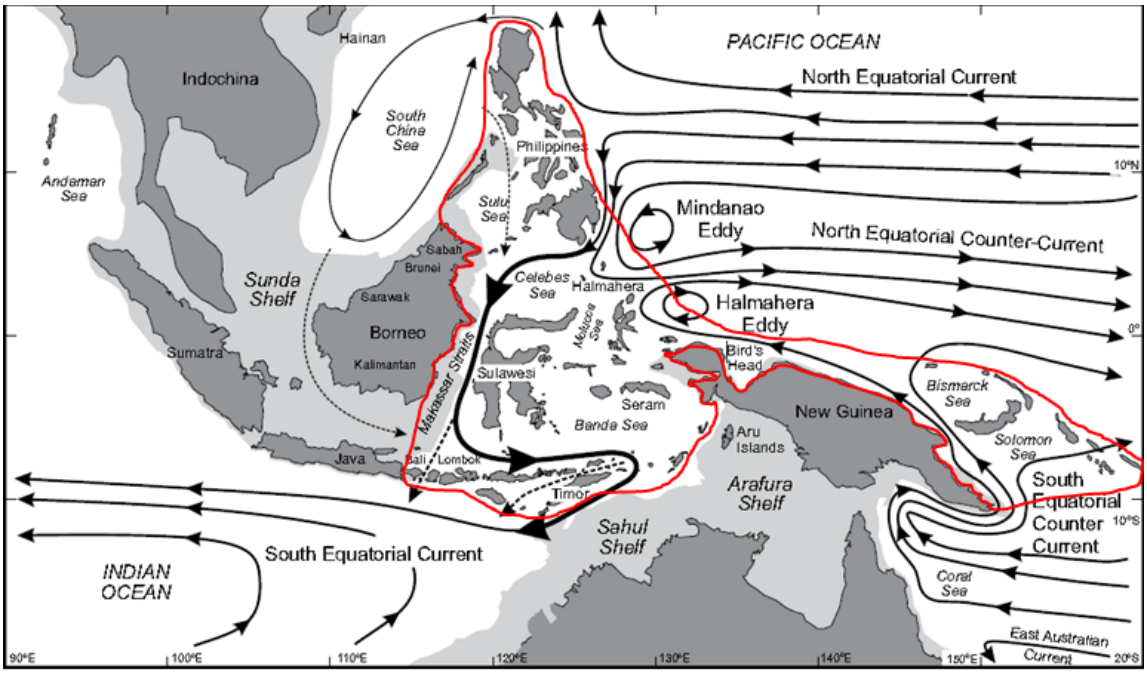

Fig. 1: The Indo-Pacific transition zone and present-day ocean currents (arrows). Land masses are indicated in dark grey and Pleistocene sea levels are indicated in light grey. The red line indicates the limits of the Coral Triangle.

The Thai-Malay Peninsula is situated on the Sunda shelf which is not part of the Coral Triangle. It was dry land during the last ice age and has been recolonized from the Pacific and the Indian Ocean when the sea level rose again (see Fig. 1 indicating Pleistocene sea level and present-day currents). Somewhere along this peninsula the

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marine biotas of the two oceans must have gotten into contact again. This contact zone may still be detectable as a genetic discontinuity (Gaither *et al.* 2011). Ocean currents can be a factor affecting gene flow by restricting dispersal. For example, the North Equatorial Pacific current (Fig. 1) arrives at the latitude of Samar island in the Philippines splitting into a northern Kuroshio current and a southern Mindanao current restricting gene flow between populations north and south of Samar. The Halmahera eddy may redirect propagules back to the reefs of West Papua, restricting gene flow to the Molucca Sea.

Phylogeography is the study of the historical processes that may be responsible for the contemporary geographic distributions of individuals (Avise *et al.* 1987). This is accomplished by considering the geographic distribution of individuals in light of the patterns associated with a gene geneology (Rua *et al.* 2011). In contrast to the flourishing development of phylogeographic studies of terrestrial organisms soon after the discipline received its name in 1987 (Avise *et al.* 1987) the approach was only applied to seaweeds rather later. The first phylogeographic study on green seaweeds was published In 1994 and the first on red algae a year later (van Oppen *et al.* 1994, 1995). In the last two decades more than 120 studies have been published employing molecular phylogeographic approaches for studying genetic diversity and evolution of seaweeds (Hu *et al.* 2016). These studies have greatly expanded our understanding of factors and processes contributing to biodiversity, adaptation, and population genetic variation of seaweeds. The vast majority of these studies was done on temperate and cold water seaweeds, predominantly from the North Atlantic. The first tropical Indo-Pacific seaweed phylogeography study was published in 2013 (Chan *et al.* 2013). Chan *et al.* (2013, 2014) studied the phylogeographical patterns of the brown algae *Sargassum polycystum* C. Agardh and *S. aquifolium* (Turner)C. Agardh using nuclear and plastidal molecular markers revealed genetic homogeneity across sites in Southeast Asia and the western Pacific, in sharp contrast to that revealed from most animal studies. The authors suggested that *Sargassum* persisted in a single refugium during the Pleistocene in a panmixia pattern. Expansion occurred more recently after the Last Glacial Maximum and recolonization of the newly flooded Sunda shelf could have involved asexual propagation of the species. High dispersal ability through floating fronds carrying developing germlings may also contribute to the low genetic diversity of the species. In contrast, Payo *et al.* (2013) found a lot of genetic variation in the red

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alga *Portieria hornemannii* (Lyngbye) Silva in the Philippines. The authors concluded that speciation in the marine realm can occur at spatial scales of less than100 km. This was a critical study clearly highlighting the potential of tropical marine algae with limited dispersal to speciate while maintaining close proximity. It is clear that morphology-based measures of biodiversity consistently underestimate that determined using molecular methods (Sherwood & Zuccarello 2016). Its implications for the study of algal phylogeography are profound. It has become evident that while “morphological” algal species appear to be widespread in the tropics, many molecularly defined species have much more limited distributions. The available data show three main trends: (1) in all studies examined the number of species discovered by molecular methods is large, (2) the increase in estimates of diversity is correlated with sampling effort, and (3) while morphological species are widespread in the tropics, cryptic or pseudo-cryptic species are often more localized, and even appear to have neighboring distribution patterns (Ni-Ni-Win *et al.* 2011, Silberfeld *et al.* 2013, Payo *et al.* 2013, Vieira *et al.* 2013, Kamiya & West 2014). In biogeography it is essential to know the species, but this is still a problem with low morphology taxa like macroalgae. Only large scale DNA barcoding campaigns can solve this. A major effort has been made sampling the red alga *Portieria hornemannii* from all over its Indo-Pacific range from Hawaii to South Africa, a collaborative research by many phycologists (Leliaert *et al.* in press). Almost 100 species were recognized based on a multi-gene sequence-based species delimitation (only seven *Portieria* species had been described). Half of the detected species occurs in the Coral Triangle.

Objectives

This project aims to explore the phylogeography of two tropical reef-associated red algae in the Indo-Pacific transition zone, to detect genetic connectivities between populations and to search for genetic discontinuities. This information is useful to define units of conservation. Target species are the enigmatic gelatinous *Gibsmithia hawaiiensis* Doty (Dumontiaceae, Gigartinales, Fig. 2) and the calcified *Dichotomaria marginata* (Ellis & Solander) Lamarck (Galaxauraceae, Nemaliales, Fig. 3).

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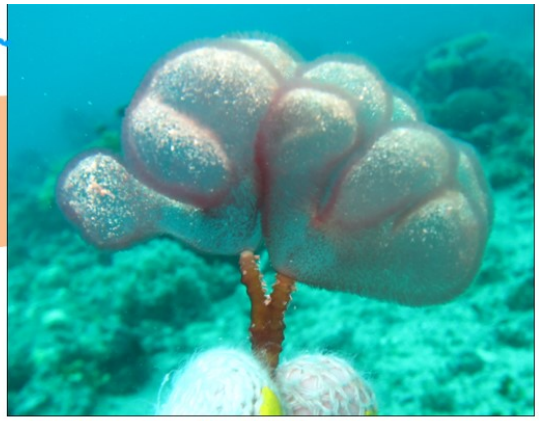
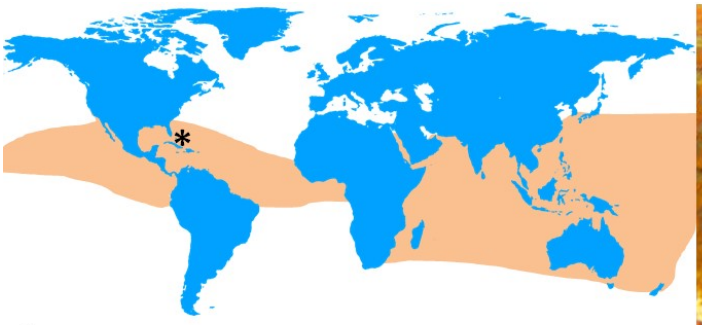


Gibsmithia hawaiiensis (Dumontiaceae, Gigartinales)

Fig. 2: Global distribution of *Gibsmithia hawaiiensis* Doty



Dichotomaria marginata (Galaxauraceae, Nemaliales)

Fig. 3: Global distribution of *Dichotomaria marginata* (Ellis & Solander) Lamarck

Methodology

Gibsmithia hawaiiensis and *Dichotomaria marginata* collections made by Dr. Stefano Draisma from 2005 onwards are housed in herbaria of the University of Malaya (Malaysia), Naturalis Biodiversity Centre (Netherlands), Tunghai University (Taiwan), and the University of Louisiana at Lafayette (USA). These collections were available for the present study and additional collections were made (Figs 4 and 5). Phylogeographic studies require a careful choice of molecular markers to be able to detect a particular effect size and appropriate temporal scale of events. Most researchers use Single Nucleotide Polymorphisms (SNPs) in non-recombining mitochondrial and chloroplast DNA sequences (Hu *et al.* 2016). It is expected to find, on average, one SNP per 200-500 sequenced non-coding base pairs and 500-1000 coding base pairs (Brumfield *et al.* 2003). The chloroplast *rbc*L gene and RuBisCO spacer and the mitochondrial CO1 gene and *cox*2,3 spacer have been used most in red algae (Sherwood *et al.* 2010).

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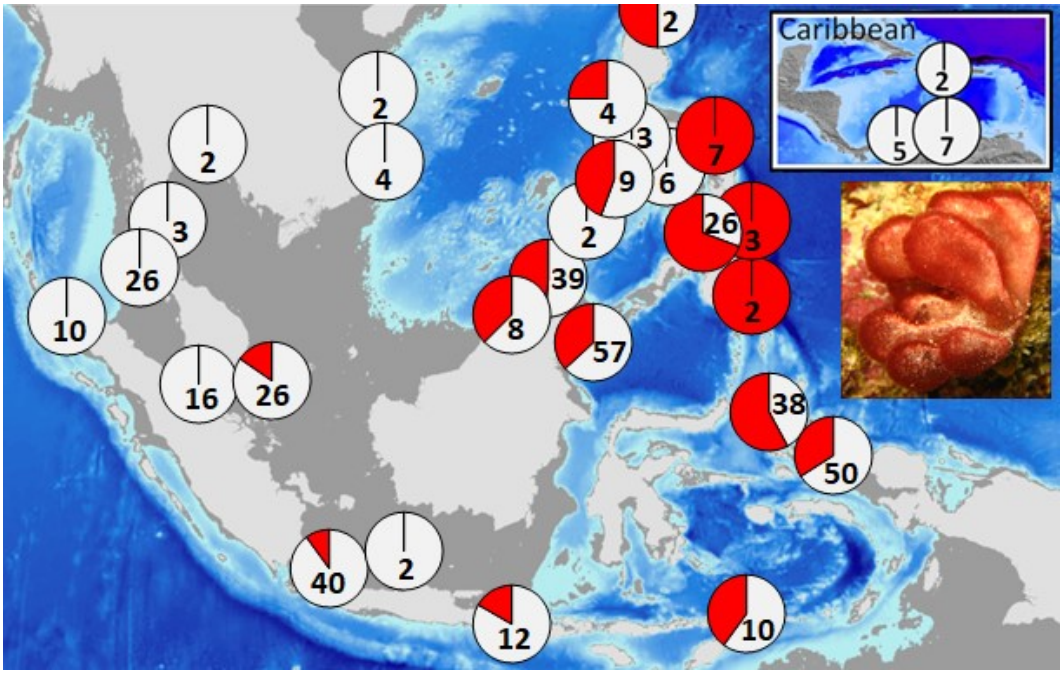

Fig. 4: *Gibsmithia hawaiiensis* sampling sites. Numbers indicate the number of dive sites visited per area. Pie charts: red represents fraction of dive sites where *G. hawaiiensis* was found, white the fraction of sites where it was not found.

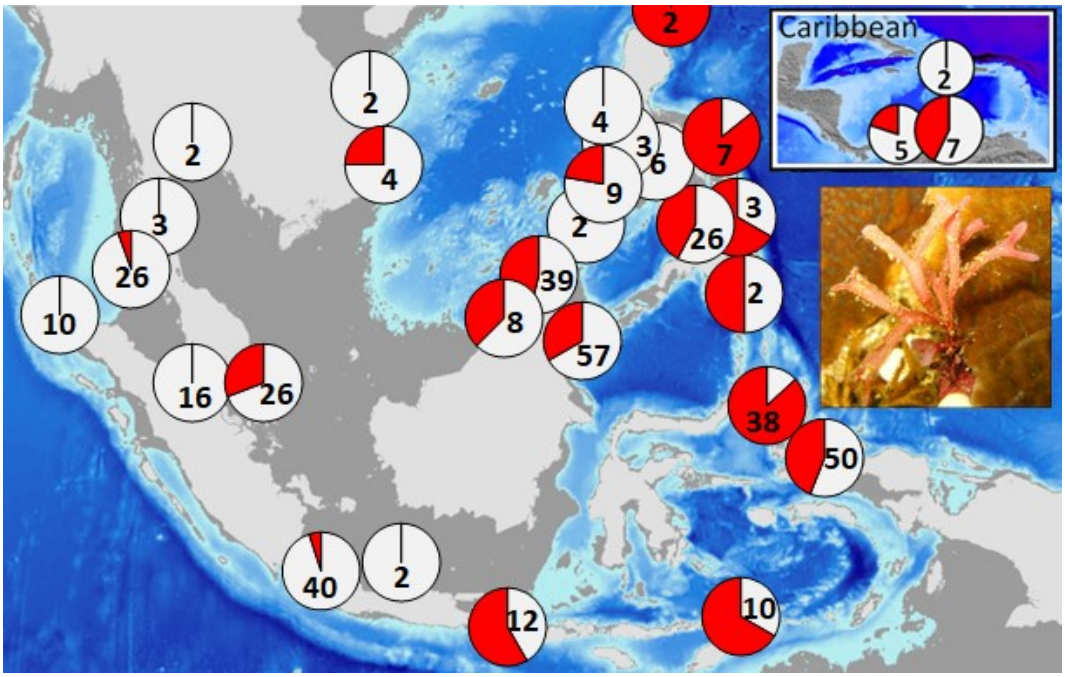

Fig. 5: *Dichotomaria marginata* sampling sites. Numbers indicate the number of dive sites visited per area. Pie charts: red represents fraction of dive sites where *D. marginata* was found, white the fraction of sites where it was not found.

Several DNA markers were targeted in search for SNPs: nuclear rDNA LSU D2-D3 region, chloroplast-encoded 5'-*rbc*L gene plus RuBisCO spacer and Universal Plastid Amplicon (UPA), and mitochondrial COI (*cox*1) and *cox*2,3 spacer (Fig. 6). Polymerase

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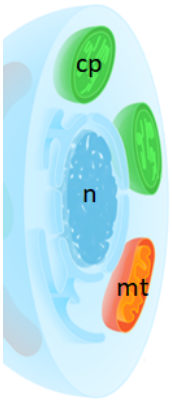
Chain Reactions followed Sherwood *et al.* (2010) and Gabriel *et al.* (2016) and sequencing was done at 1st BASE DNA Sequencing Services (Malaysia). Sequences were aligned in BioEdit (Hall 1999) which was also used for phylogenetic inference using the Maximum Likelihood (ML) algorithm. Haplotype networks were reconstructed using TSC (Clement *et al.* 2000).



	Dichotomaria marginata				*Gibsmithia malayensis*			
marker	length (bp)	# samples	# SNPs	% var. sites	length (bp)	# samples	# SNPs	% var. sites
*rbc*LS	890	n=68	15	1.7%	870	n=22	4	0.5%
*rbc*L-3'	800	n=89	14	1.7%	1280	n=10	5	0.4%
*rbc*L	1370	n=25	19	1.4%	1330	n=7	5	0.4%
UPA	370	n=9	1	0.3%	370	n=23	2	0.5%
LSU D2-D3	600	n=9	*3*	*0.5%*	580	n=6	*2*	*0.3%*
*cox*1-5'	520	n=28	43	8.3%	-	-	-	-
*cox*1	1390	n=5	62	4.5%	-	-	-	-
*cox*1-3'	850	n=10	33	3.9%	780	n=6	0	0.0%
*cox*2-3 sp.	300	n=18	24	8.0%	380	n=48	11	2.9%

Fig. 6: Tested DNA markers and their variability in *Dichotomaria marginata* and *Gibsmithia malayensis*. Background colors represent cellular compartments indicated on the left (cp, chloroplast; n, nucleus; mt, mitochondrion.

Results and Discussion

Genetic variation for each marker was lower in *Gibsmithia* than in *Dichotomaria* (Fig. 6), possibly indicating more recent divergence of the former taxon. Based on variability and amplifyability, 3'-*rbc*L was chosen as genetic marker for *Dichotomaria* and *cox*2,3 for *Gibsmithia*. DNA sequence data revealed cryptic diversity in both taxa.

Gibsmithia

An rbcL-based Maximum Likelihood (ML) phylogeny of *Gibsmithia* is shown in Figure 7 and species distribution in Southeast Asia Figure 8.

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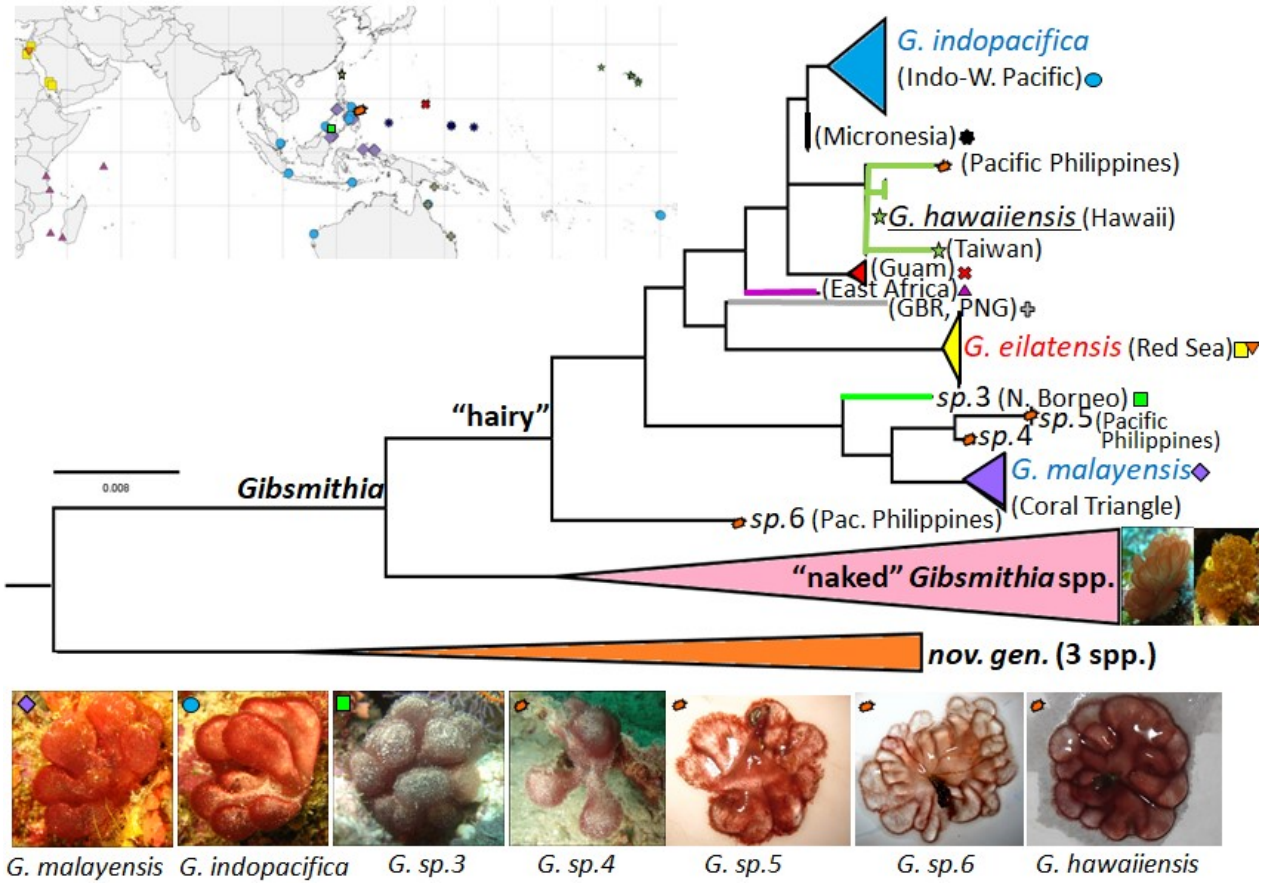

Fig. 7: *rbc*L Maximum Likelihood phylogeny of the genus *Gibsmithia*. Size of triangles is proportional to the number of sequences used in the analysis. The outgroup (genus *Dudresnaya* P. Crouan & H. Crouan) was pruned after analysis. Specimens' origins are indicated on the map (upper left). Habits of representative specimens are shown at the bottom.

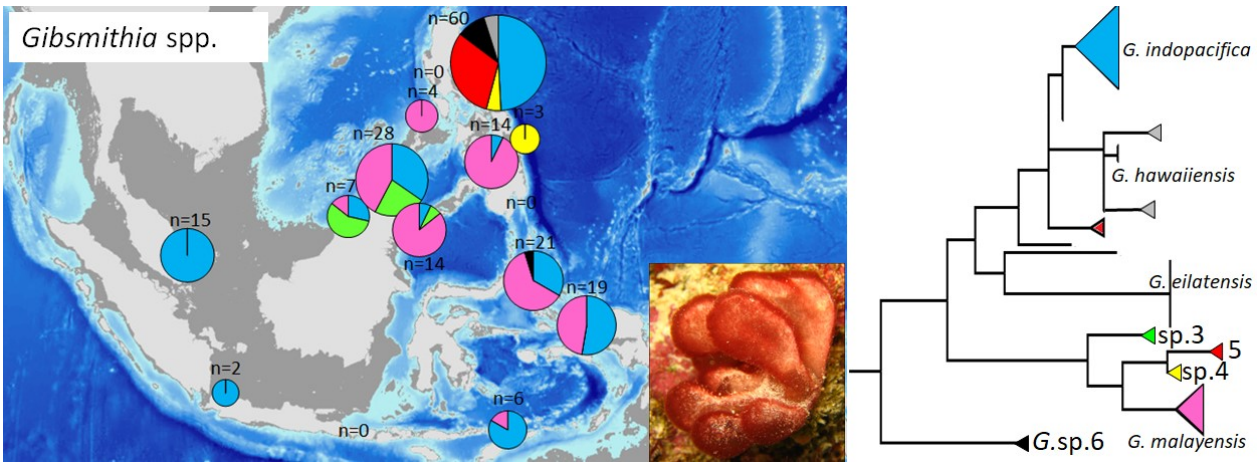

Fig. 8: Distribution of hairy *Gibsmithia* species in Southeast Asia (ML phylogeny from Fig. 7 right)

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Gibsmithia hawaiiensis (type location Hawaii) was only found in Taiwan and the Pacific coast of the Philippines, albeit genetically different from each other and from Hawaiian populations, likely as a result of isolation-by-distance. These population may be in the process of speciation. One *Gibsmithia* species is widely distributed in Southeast Asia, whereas another species is also widely distributed but restricted to the Coral Triangle. These two species are newly described as, respectively, *G. indopacifica* D. Gabriel, Draisma & Fredericq and *G. malayensis* D. Gabriel, Draisma & Fredericq (Gabriel *et al.* 2017). However, in practice these are cryptic species as they are not distinguishable from each other and from *G hawaiiensis* based on their macro-morphology (external habit). Species descriptions are based on microscopic reproductive structures. Four more cryptic *Gibsmithia* species were found, one around the northern tip of Borneo (sp. 3) and three on the Philippine Pacific coast (sp. 4, 5, 6). These four species remain to be studied and possibly more collections are needed to find enough reproductive specimens and it cannot be guaranteed that diagnostic characters will be discovered. The Pacific Philippines appears to be the centre of *Gibsmithia* diversity with five out of seven Southeast Asian species (*G. malayensis* was not found here). Dr. Daniela Gabriel discovered more (undescribed) *Gibsmithia* species outside Southeast Asia (see Fig. 7), but each confined to a geographic area and not living in sympatry with a congeneric. The unique *cox*2,3 haplotype of the Philippine Pacific population of *G. indopacifica* emerges in the centre of a haplotype network (Fig. 9), hinting that this may be a centre of origin of this species. The Pacific Philippines could also be a centre of *Gibsmithia* speciation, rather than a centre of species accumulation or overlapping distributions, because three species were only found in the Philippine Sea of which two are closely related sister-species (spp. 4 and 5). There appears to be low connectivity between *G. indopacifica* and *G. malayensis* populations from the South China Sea (*G. indopacifica* only), Sulu Sea, Molucca Sea, and Halmahera Sea (Fig. 9), but more specimens need to be haplotyped for a clearer picture. Seven “hairy” *Gibsmithia* species (with filaments protruding the surface of a gelatinous matrix) are recognized in Southeast Asia and they form a monophyletic clade (Fig. 7). The sister clade of the “hairy” *Gibsmithia* clade consists of “naked” *Gibsmithia* species (filaments not protruding) consisting of three described species and several undescribed species (unpublished). Moreover, Dr. Daniela Gabriel and Dr. Stefano Draisma simultaneously discovered a new sister-genus of *Gibsmithia* from respectively the Gulf of Mexico and Southeast Asia (unpublished).

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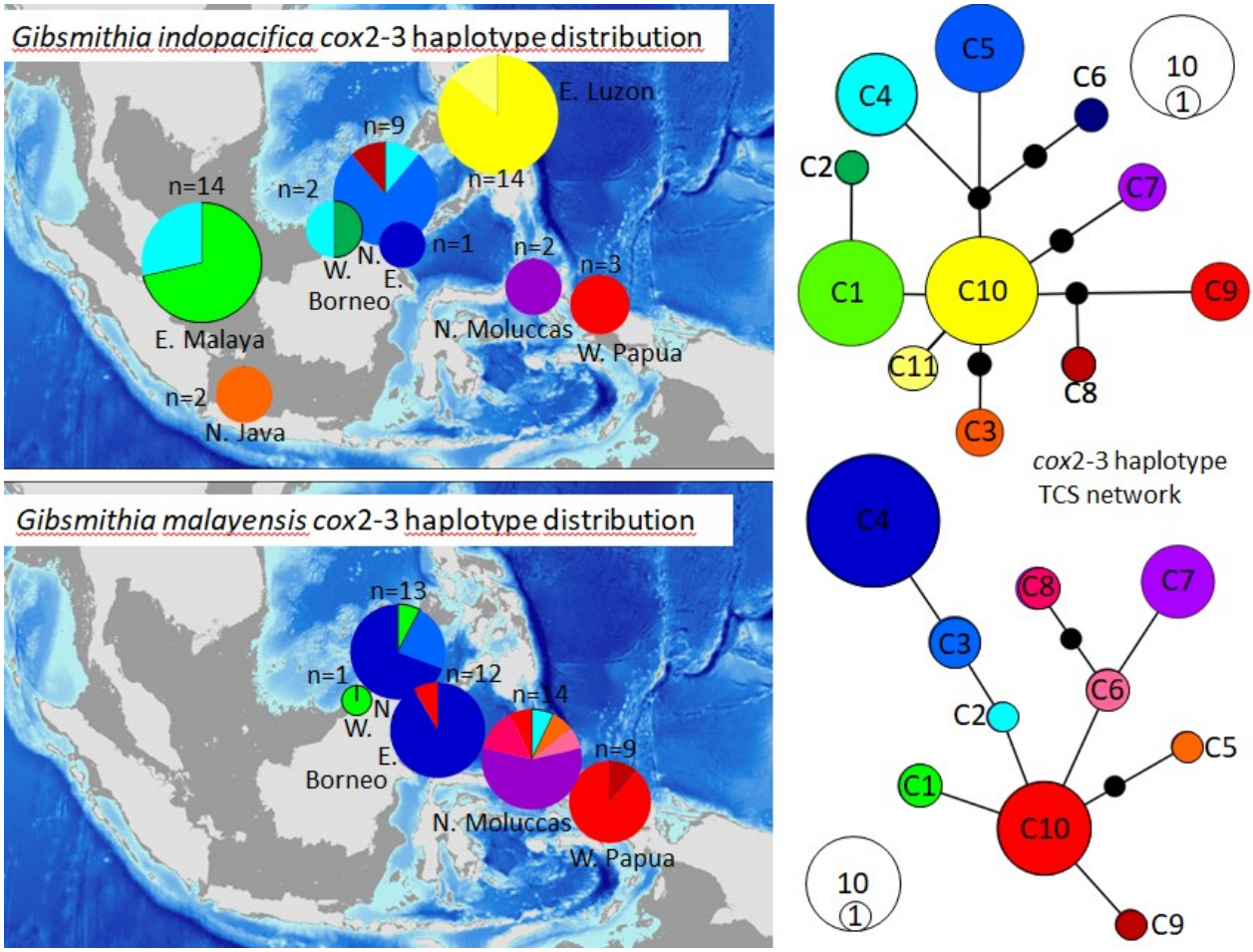

Fig. 9: *cox*2,3 haplotype networks (right) of, respecitively, *Gibsmithia indopacifica* (top, haplotpyes C1-C11) and *G. malayensis* (bottom, haplotypes C1-C10). Circle size represents sample number. Distribution of haplotypes in Southeast Asia (left)

Dichotomaria

An rbcL-based ML phylogeny of *Dichotomaria* is shown in Figure 10 and the distribution of the genotyped samples in the present study in Figure 11. The type location of *D. marginata* is in the Caribbean, but DNA sequence data indicate the occurrence of two flattened *Dichotomaria* species in the Caribbean, of which one is also widespread in Southeast Asia. At this moment we are not sure which of the two is the true *D. marginata*, but two varieties have been described from the Caribbean and one could be given species status in the future (Wynne 2005). For the time being we use the name *D. marginata* for the most common and widely distributed *Dichotomaria* in Southeast Asia. An unknown flattened *Dichotomaria* species (sp. 4) was found in Borneo (9 specimens). It has been previously reported from Luzon, Philippines (Wiriyadamrikul *et al.* 2014). Another unknown *Dichotomaria* species (sp. 7) was found in Nusa Tenggara, Indonesia (2 specimens).

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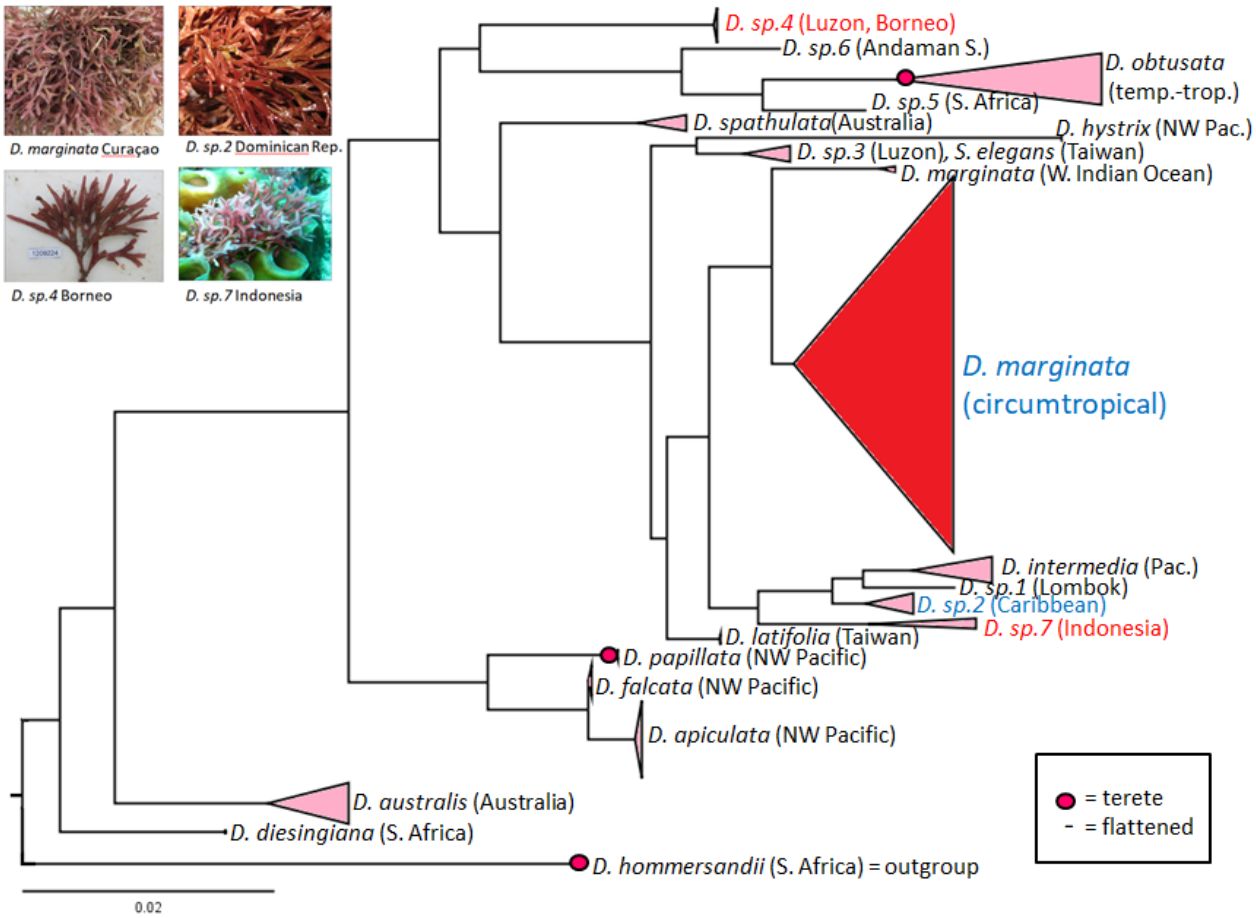

Fig. 10: *rbc*L Maximum Likelihood phylogeny of the genus *Dichotomaria*. Triangle size is proportional to sample size. Blue and red taxon labels were found int he present study. Small red circles indicate taxa with terete (cylindrical) branches. All other species have flattened branches. Habits of four representative specimens shown upper left. Species numbering 1-6 follows Wiriyadamrikul *et al.* (2014).

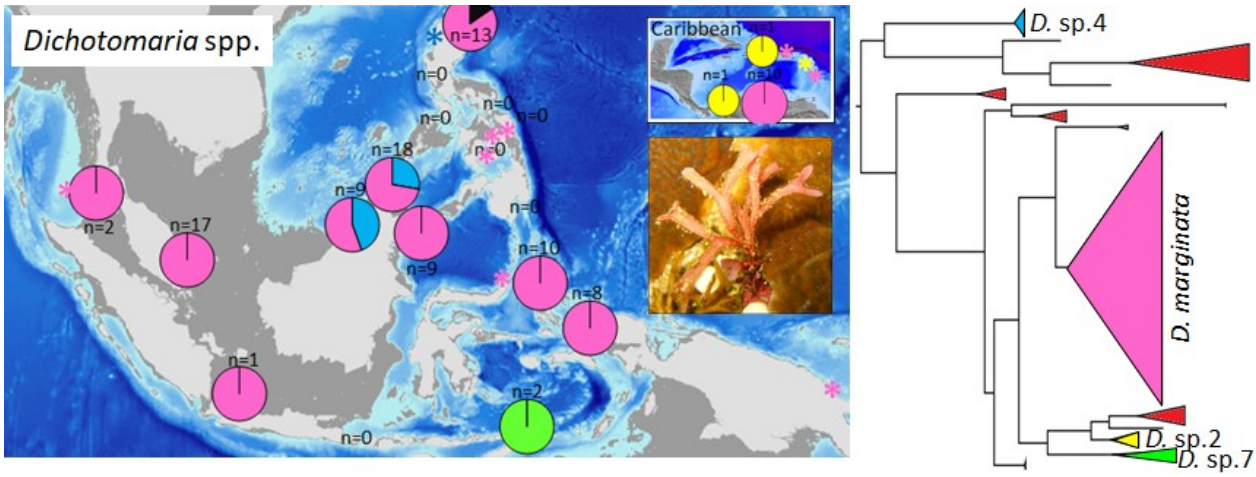

Fig. 11: Distribution of flattend *Dichotmaria* in Southeast Asia and the Caribean (inset) found in the present study (ML phylogeny from Fig. 10 right). The black fraction in Taiwan represents *D. latifolia*. Asterisks (*) indicate records in Wiriyadamrikul *et al.* (2014).

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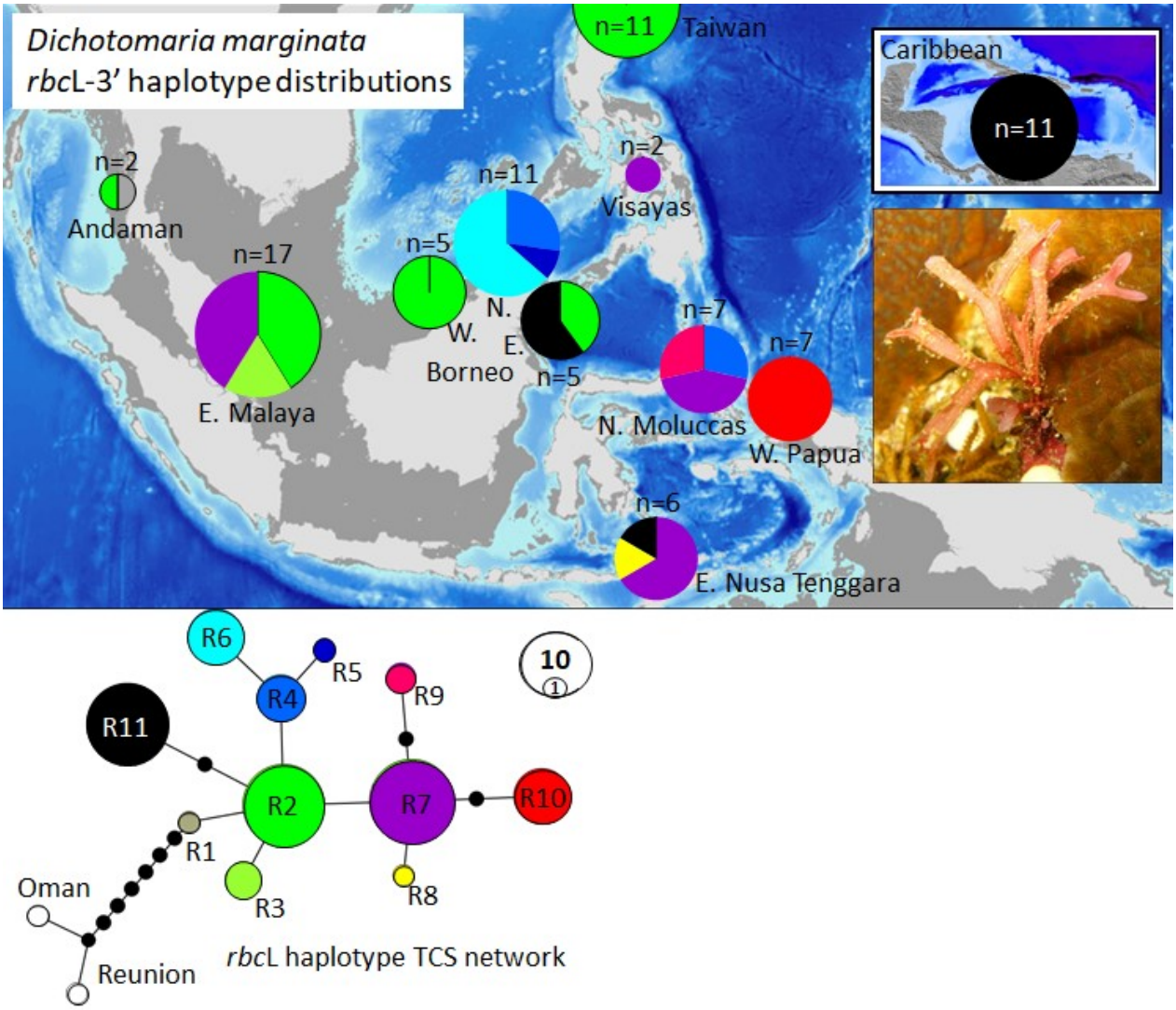

Fig. 12: 3'-*rbc*L haplotype network (bottom, haplotypes R1-R11) of *Dichotomaria marginata*. Circle size represents sample size. Distribution of haplotypes R1-R11 in Southeast Asia (top) and the Caribbean (inset top right). Two unnamed haplotypes from Oman and Reunion are indicated in the network, but not on the map.

The *D. marginata* 3'-*rbc*L haplotype distribution pattern (Fig. 12) indicates that there is probably higher connectivity in this species compared to *Gibsmithia* spp. as two haplotypes (*i.e.*, R2 and R7, both central in a haplotype network) are found all over Southeast Asia, except in the Halmahera Sea which again appears to be isolated, likely as a consequence of the Halmahera eddy impeding long distance dispersal. The Philippine Sea specimens have yet to be haplotyped, but samples from southern Taiwan all represented one of the common haplotypes (R2). Two western Indian Ocean *D. marginata* specimens (from Oman and Reunion) are distantly related to Southeast Asian specimens, but considered conspecific. However, the Caribbean 3'-*rbc*L haplotype (R11), was also found in Southeast Asia. However, Southeast Asian and Caribbean

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populations with R11 3'-*rbc*L haplotypes differ in 5'-*rbc*L. Still it is difficult to explain that Caribbean populations are more closely related to Southeast Asian populations than western Indian Ocean populations are to Southeast Asian populations.

Dichotomaria is one of four genera in the family Galaxauraceae, the other three being *Actinotrichia*, *Galaxaura*, and *Tricleocarpa*. Collaborator Ass. Prof. Dr. Hsao-Lun Liu at Tunghai University (Taichung, Taiwan) assembled a DNA sequence data set (unpublished) of Indo-Pacific Galaxauraceae revealing that the actual number of species in each genus is significantly higher than the number of currently accepted species in AlgaeBase (www.algaebase.org), except for the genus *Galaxaura* (possibly due to heteromorphic species). Most Galaxauraceae have a restricted distribution and only a few species exhibit a range across the Indo-Pacific, again with the exception of the *Galaxaura* species which are predominantly wide-spread. Southeast Asia appears to be the centre of Galaxauraceae diversity, but it should be noted that this was also the most intensively sampled region and therefore this conclusion might turn out to be premature. However, it is congruent with other tropical marine taxa. Our preliminary results appear to corroborate earlier findings for shallow water invertebrates and fish (Carpenter & Springer 2005) and highlight the current unstable taxonomy of tropical red algae. Future research needs to focus on haplotyping more specimens per population and using more markers to get a clearer picture of genetic connectivities in Southeast Asia. Furthermore, the search goes on for diagnostic morphological characters to describe the newly recognized phylogenetic species. We embarked on a collaborative project with colleagues from the Institute of Oceanology in Qingdao (China) to study the phylogeography of the brown algae *Sargassum polycystum* and *Padina boryana*, and the green alga *Halimeda macroloba* using Genotyping-by-Sequencing-based population genomics.

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5. Output จากโครงการวิจัยที่ได้รับทุนจาก สกว.

1. ผลงานตีพิมพ์ในวารสารวิชาการนานาชาติ (ระบุชื่อผู้แต่ง ชื่อเรื่อง ชื่อวารสาร ปี เล่มที่ เลขที่ และหน้า) พร้อมแจ้งสถานะของการตีพิมพ์ เช่น

Gabriel, D., Draisma, S.G.A., Schmidt, W.E., Schils, T., Sauvage, T., Maridakis, C., Gurgel, C.F.D., Harris, D.J. & Fredericq, S. Beneath the hairy look: The hidden reproductive diversity of the *Gibsmithia hawaiiensis* complex (Dumontiaceae, Rhodophyta). 2017 *Journal of Phycology* 53: 1171-1192. DOI: 10.1111/jpy.12593.

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2. การนำผลงานวิจัยไปใช้ประโยชน์

- เชิงพาณิชย์ (มีการนำไปผลิต/ขาย/ก่อให้เกิดรายได้ หรือมีการนำไปประยุกต์ใช้โดยภาคธุรกิจ/บุคคลทั่วไป)
- เชิงนโยบาย (มีการกำหนดนโยบายอิงงานวิจัย/เกิดมาตรการใหม่/เปลี่ยนแปลงระเบียบข้อบังคับหรือวิธีทำงาน)
- เชิงสาธารณะ (มีเครือข่ายความร่วมมือ/สร้างกระแสความสนใจในวงกว้าง)
- เชิงวิชาการ (มีการพัฒนาการเรียนการสอน/สร้างนักวิจัยใหม่)

3. อื่นๆ (เช่น ผลงานตีพิมพ์ในวารสารวิชาการในประเทศ การเสนอผลงานในที่ประชุมวิชาการ หนังสือ การจดสิทธิบัตร)

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BENEATH THE HAIRY LOOK: THE HIDDEN REPRODUCTIVE DIVERSITY OF THE *GIBSMITHIA HAWAIIENSIS* COMPLEX (DUMONTIACEAE, RHODOPHYTA)[1]

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The tropical alga previously recognized as *Gibsmithia hawaiiensis* (Dumontiaceae, Rhodophyta) was recently suggested to represent a complex of species distributed throughout the Indo-Pacific Ocean and characterized by a peculiar combination of hairy (pilose) gelatinous lobes growing on cartilaginous stalks. Phylogenetic reconstructions based on three genetic markers are presented here with the inclusion of new samples. Further diversity is reported within the complex, with nine lineages spread in four major phylogenetic groups. The threshold between intra- and interspecific relationships was assessed by species delimitation methods, which indicate the existence of 8–10 putative species in the complex. Two species belonging to the *G. hawaiiensis* complex are described here: *Gibsmithia malayensis* sp. nov. from the Coral Triangle and *Gibsmithia indopacifica* sp. nov., widely distributed in the Central and Eastern Indo-Pacific. Morphological differences in the vegetative and reproductive structures of the newly described species are provided and compared to the previously described species of the complex. Additional lineages represent putative species, which await further investigation to clarify their taxonomic status. *Gibsmithia hawaiiensis* sensu stricto is confirmed to be endemic to the Hawaiian Islands, and *Gibsmithia eilatensis* is apparently confined to the Red Sea, with an expanded distribution in the region. New records of the *G. hawaiiensis* complex are reported from Egypt, Saudi Arabia, Indonesia, Philippines, and the Federated States of Micronesia, indicating that the complex is more broadly distributed than previously considered. The isolated position of

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***Gibsmithia* within the Dumontiaceae is corroborated by molecular data.**

***Key index words:* COI-5P; cryptic species; DNA barcode; Dumontiaceae; *Gibsmithia hawaiiensis* complex; phylogenetics; *rbc*L; species delimitation methods; taxonomy; UPA**

***Abbreviations*: ABGD, Automated Barcode Gap Discovery method; BI, Bayesian Inference; COI-5P, cytochrome c oxidase subunit 1, 5′-prime end; GMYC, Generalized Mixed Yule Coalescent model; ML, Maximum Likelihood; PTP, Poisson Tree Process model; *rbc*L, ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit; SDM, species delimitation methods; SPN, Statistical Parsimony Network analysis; UPA, plastid LSU (23S) domain V**

The Dumontiaceae (Gigartinales) represent a family of red algae characterized by separate carpogonial and auxiliary cell branches, with distinctive postfertilization events (Tai et al. 2001). Such events include (i) the fusion of a fertilized carpogonium (or its products) with other cells of the carpogonial branch before issuing connecting filaments, (ii) the division of those filaments before attaching to an auxiliary cell, and (iii) the development of the gonimoblast initials from the part of the connecting filament fused with the auxiliary cell (Lindstrom 1984, figs. 13.21–13.26, p. 315, in Hommersand and Fredericq 1990).

Despite these distinctive post-fertilization events, members of the Dumontiaceae exhibit "perhaps the greatest morphological diversity seen in any red algal family" (Robins and Kraft 1985), including uniaxial and multiaxial genera, with or without secondary pit-connections, from foliose to laxly filamentous plants. In addition, some significant variation related to reproduction is found, such as the fusion of the auxiliary cell with other cells of the auxiliary cell branch in some genera, tetrasporophytes being isomorphic or heteromorphic with gametophytes, and tetrasporophytes with zonate, cruciate or irregularly divided tetrasporangia (Robins and Kraft 1985).

Besides the explicit diversity, genetic studies have unveiled the existence of cryptic species (i.e., "species lacking obvious morphological diagnostic features that are classified as a single nominal species"; Pante et al. 2015), within some cold-water genera in the Dumontiaceae (Tai et al. 2001, Saunders 2005). For instance, using DNA barcoding to assist alpha taxonomy, Saunders (2008) highlighted the existence of overlooked morphological and anatomical variations among Canadian taxa, while Saunders and Lindstrom (2011) applied a multigene phylogeny to assess the diversity within the *Dilsea/Neodilsea* complex in the northeastern Pacific.

Among tropical members of the family, Gabriel et al. (2016) recently investigated the *Gibsmithia hawaiiensis* complex, a peculiar taxon characterized by ephemeral clusters of "hairy" gelatinous lobes arising from perennial, cartilaginous stalks (Fig. 1). Based on three genetic markers (mitochondrial COI-5P, and plastid *rbc*L and UPA), Gabriel et al. (2016) suggested that *G. hawaiiensis* represents a species complex comprising five distinct lineages: the generitype from the Hawaiian archipelago (Fig. 1A), the newly described *Gibsmithia eilatensis* from the Red Sea (Fig. 1, B and C), and three undescribed species occurring in different regions of the Indo-Pacific.

Recent collections from Southeast Asia and the Red Sea, as well as additional loans from the West Indian Ocean, the Red Sea and Australia allowed further research of the genetic variation and anatomical explorations of fertile specimens in the *Gibsmithia hawaiiensis* complex. The present work combines multigene phylogenies (based on *rbc*L, COI-5P and UPA), species delimitation methods (SDMs) and morphological appraisal to further assess the diversity of the *G. hawaiiensis* complex.

MATERIAL AND METHODS

Sampling and specimen processing. Samples collected by SCUBA diving or snorkeling were fixed in 4% formalin-seawater or pressed on herbarium paper, with subsamples preserved in silica gel or 96% ethanol for further molecular studies. Whenever possible, underwater pictures were taken to illustrate the specimens' habit and habitat. Newly collected samples were deposited in the University of Malaya Herbarium (KLU), the University of Louisiana at Lafayette Herbarium (LAF), and the National Herbarium of the Netherlands (L). Additional samples were obtained through loans from the Ghent University Herbarium (GENT) and the State Herbarium of South Australia (AD), and contributions from various researchers. The identification of *Gibsmithia* species was primarily based on the studies from Kraft (1986) and Gabriel et al. (2016). The total collection consisted of 64 new samples from various locations in the Indo-Pacific (Table S1 in the Supporting Information; Fig. 2).

Morphological studies. Small fragments of formalin-preserved specimens were stained with 4% aqueous aniline blue on microscope slides for 15 min, following the procedure for gelatinous taxa of Gabriel et al. (2009). After washing with distilled water, the stained fragments were mounted in a 50% Karo/water solution and squashed under the glass cover slip. The permanent slides were examined using an optical microscope (Leica DM2500, Wetzlar, Germany) with a microphotography system (Leica LAS V3.8), with which measurements (presented as length × width) and photographs were made. All pictures were edited in Photoshop v.12 (Adobe Systems Incorporated, San Jose, CA, USA).

DNA extraction, amplification and sequencing. Subsamples preserved in ethanol (after air-drying) or in silica gel were ground with mortar and pestle, and total DNA was extracted using DNeasy Plant Mini Kits (Qiagen, Valencia, CA, USA) following the manufacturer's instructions. DNA from specimens dried on herbarium paper was extracted using a modified Dellaporta et al. (1983) protocol described by Hughey et al. (2001).

DNA sequences were generated for three genetic markers: the mitochondrial encoded COI-5P (cytochrome c oxidase subunit 1, 5′-prime end, DNA barcode region, 664 bp), and the chloroplast encoded *rbc*L (ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit, 1,467 bp) and UPA (plastid LSU (23S) domain V, 377 bp). The amplification

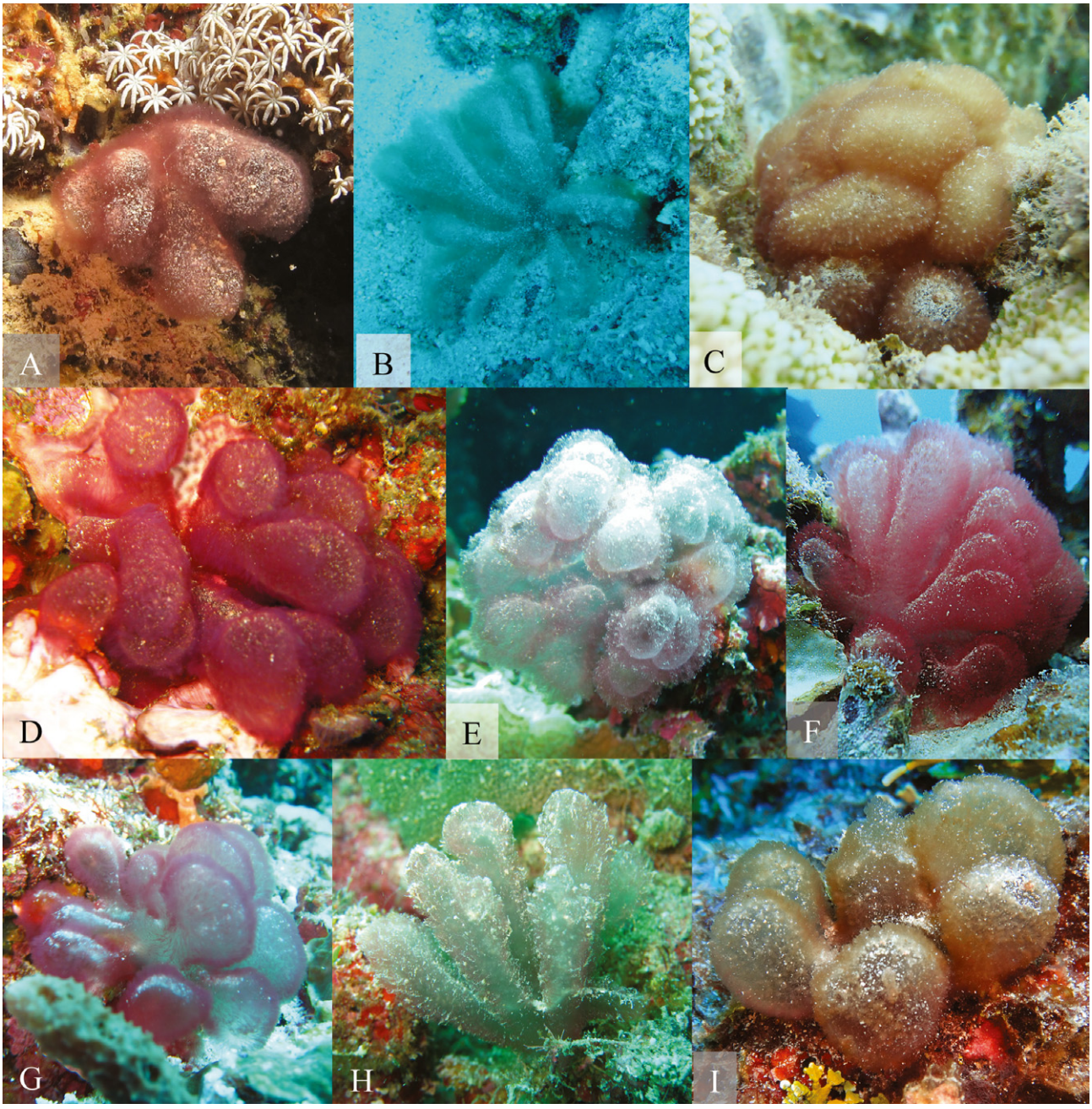

FIG. 1. In situ habits of *Gibsmithia hawaiiensis* complex from different locations. (A, B) *Gibsmithia eilatensis* (lineage E). (A) Farasan Banks, Saudi Arabia; (B) Hurghada, Egypt. (C) *Gibsmithia* sp. 4 (lineage H), Sharm El-Sheik, Egypt. (D, E) *Gibsmithia malayensis* sp. nov. (lineage D). (D) Ternate, Indonesia; (E) Moalboal, Philippines. (F) *Gibsmithia* sp. 3 (lineage G), Guam. (G, H) *Gibsmithia indopacifica* sp. nov. (lineage B). (G) Nusa Tenggara Timur, Indonesia; (H) Johor, Malaysia. (I) *Gibsmithia* sp. 5 (lineage I), Pohnpei, Federate States of Micronesia. [Color figure can be viewed at wileyonlinelibrary.com]

reactions for COI-5P (except for the Australian specimens) used different combinations of the primers GazF1 and GazR1 (Saunders 2005), R686 (Sherwood et al. 2010), and cox59F, cox176F and cox606R (Gabriel et al. 2016); *rbc*L, amplified in two fragments, used the pair of primers F15-R916 and F645-R1389 (Gabriel et al. 2016, Lin et al. 2001), and UPA used the primers p23Sv_f1 and p23Sv_r1, following the amplification conditions described by Saunders and Moore (2013). PCR products were gel-purified or cleaned with ExoSAP-it (Affymetrix, USB, Cleveland, OH, USA) and submitted to DNA sequencing reactions, with the same set of PCR primers, using the BigDye Terminator v. 3.1 Cycle Sequencing Kit (Life Technologies Corporation, Carlsbad, CA, USA). Cycle sequencing reactions were purified with Sephadex G-50 Fine (GE Healthcare, Buckinghamshire, UK) and sequenced in-house on an ABI 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). Bi-directional reads were used to generate consensus sequences using Sequencher v. 5.2 (Gene Codes Corporation, Ann Arbor, MI, USA). COI-5P sequences from Australian specimens were generated according to

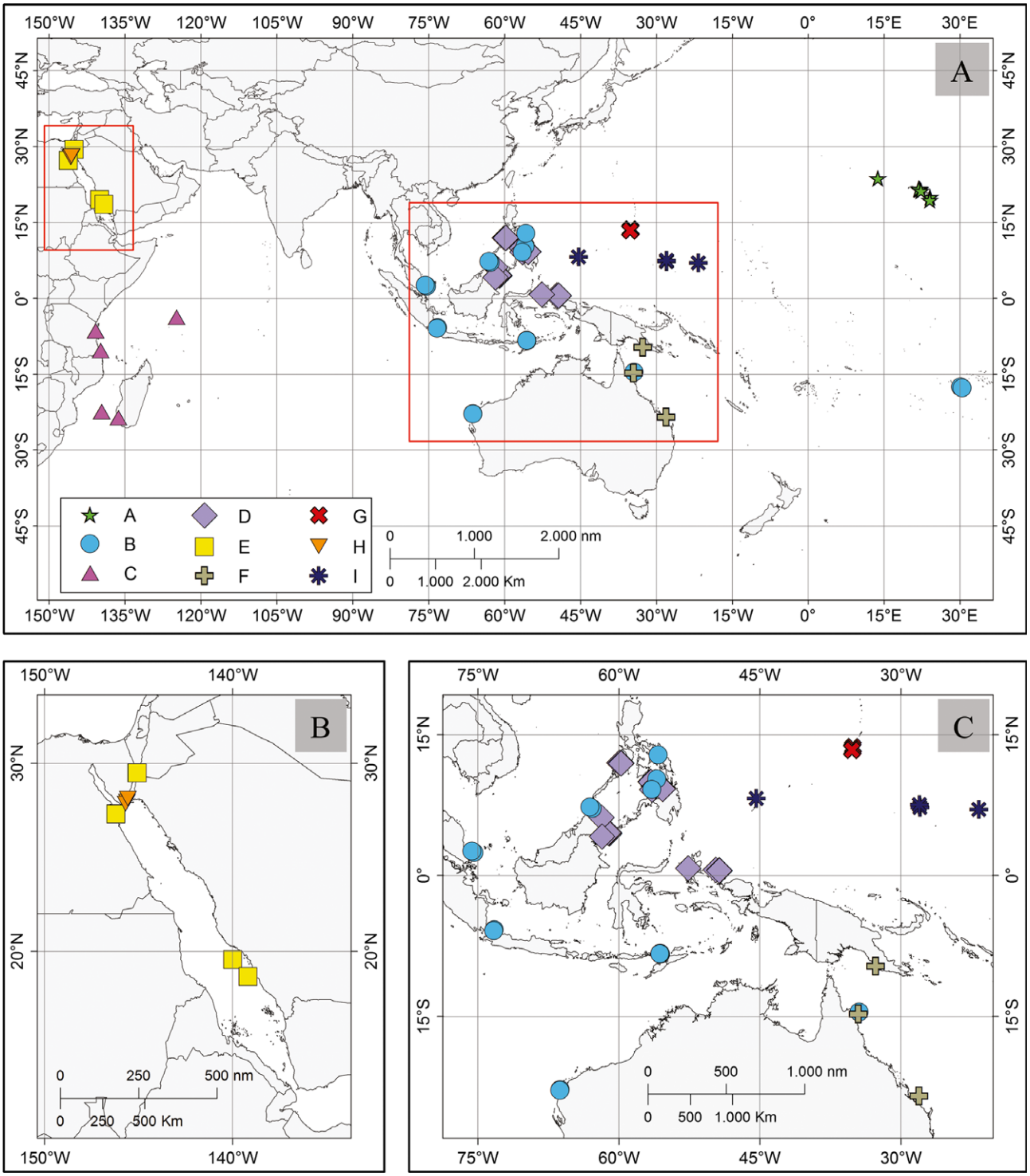

FIG. 2. Sample locations of the studied specimens belonging to the *Gibsmithia hawaiiensis* complex. Letters A–I represent the different mitochondrial lineages as marked in Figure 3. (A) Map of the Indo-Pacific basin. (B) Close up of the Red Sea (note sympatric distribution of lineages E and H). (C) Close up of the Central Indo-Pacific (note the sympatric distribution of lineages B, D and F). [Color figure can be viewed at wileyonlinelibrary.com]

Gulbransen et al. (2012). All new sequences are deposited in GenBank (Table S1).

Sequence analyses. The newly generated COI-5P, *rbc*L and UPA sequences (18, 44, and 47, respectively) were aligned and manually edited in MEGA v. 7.0.18 (Kumar et al. 2016). The coding sequences of COI-5P and *rbc*L were translated in MEGA and checked for errors or presence of premature stop codons. Additional sequences (40 COI-5P, 56 *rbc*L and 34 UPA) were downloaded from the public NCBI database GenBank and added to each dataset.

After trimming the ends so that all sequences had no missing bases, COI-5P sequences consisted of 576 bp, *rbc*L of 1,057 bp, and UPA of 321 bp. 31 *rbc*L sequences with extensive missing data were removed from the dataset. The final COI-5P, *rbc*L and UPA datasets comprised a total of 58, 69, and 81 sequences, respectively, including specimens belonging to the *Gibsmithia hawaiiensis* complex, *Gibsmithia dotyi*, *Gibsmithia larkumii*, other genera within the Dumontiaceae and one outgroup (Table S1). A selection of publicly available (GenBank) and new DNA sequences (unpubl. data) belonging to other members of the family were included to improve phylogenetic inference (Pollock et al. 2002). The selection was made to include the largest number of genera possible, with species represented by all three genes. *Euthora cristata* in the Kallymeniaceae, which is the family most closely related to the Dumontiaceae following Yang et al. (2016), was used as outgroup.

Sequence divergences were estimated using uncorrected *p*-distance as implemented in MEGA. The number of variable sites and parsimony-informative sites was computed using PAUP 4.0a151 (Swofford 2002). Haplotype identification, haplotype diversity (Hd) and nucleotide diversity (π) estimates were conducted in DnaSP ver. 5.10.01 (Librado and Rozas 2009).

To assess the congruence of independently evolving markers (Leliaert et al. 2014), phylogenetic inferences were conducted for each marker separately. Due to the extensive lack of overlapping sequences, the genetic markers were not concatenated to produce a single multigene tree.

The most appropriate DNA substitution model determined by jModelTest 2.0 v. 2.1.6 (Darriba et al. 2012), using the Akaike information criterion, for each gene was: GTR+I+G for COI-5P, TIM1+I+G for *rbc*L, and TIM1+G for UPA. ML analyses were conducted in RAxML v.8 (Stamatakis 2014) using the GTR+I+G model and the default rapid hill-climbing algorithm. Nodal support was estimated using a nonparametric bootstrap estimate with 1,000 replicates and a random starting tree. Bootstrap values (BS) were obtained via a consensus tree under the 50% majority-rule computed in PAUP.

Bayesian Inference reconstructions were performed in MrBayes v. 3.2.6 (Ronquist et al. 2012), using ten million generations with two independent runs of four Monte Carlo Markov chains (MCMC), sampled every 1,000 generations. After discarding the initial 20% generations as burn-in, the remaining trees were used to construct the 50% majority-rule consensus tree to estimate the Bayesian posterior probabilities (PP).

jModelTest, RAxML, and MrBayes were implemented at the CIPRES Science Gateway (https://www.phylo.org/; Miller et al. 2010). BS greater than 80% and PP greater than 0.95 were considered strong support (Erixon et al. 2003).

A neighbor-joining analysis was performed in MEGA, using the *p*-distance method, for an alignment with all *rbc*L sequences belonging to *Gibsmithia* (including the 31 incomplete sequences previously removed). This analysis was conducted to assign the specimens not used in the phylogenetic inferences to their respective *rbc*L genetic lineages.

For the short UPA barcode sequences, a median-joining network was also created for the *Gibsmithia hawaiiensis* complex in PopART (Leigh and Bryant 2015).

Species delimitation methods. The proposed DNA barcode for red algae, i.e., COI-5P (Saunders 2005, 2008, Sherwood et al. 2010), was selected for the single-marker SDMs, following Gabriel et al. (2016). This study reported that the sequence divergences were higher and genetic gaps between clades of *Gibsmithia hawaiiensis* were greater for COI-5P than for *rbc*L and UPA, both important criteria when choosing a marker for SDM (Leliaert et al. 2014). Since each SDM makes different simplifying assumptions, a wide range of methods was applied to the selected dataset. Congruence across methods was assessed in a conservative approach to infer putative species (Carstens et al. 2013).

Statistical Parsimony Networks (SPN) use pairwise sequence distances to generate haplotype networks, and then consider unconnected singletons or networks as distinct species (Hart and Sunday 2007). The SPN analysis was conducted in TCS v.1.21 (Clement et al. 2000) with connection limits of 95% (Hart and Sunday 2007) and 99% (Lyra et al. 2016).

The Automated Barcode Gap Discovery (ABGD) detects the barcode gap, i.e., the break in the distribution of genetic pairwise distances (Hebert et al. 2003), without a priori species hypotheses. The method then uses the observed gap to sort sequences into putative species instead of using a fixed threshold of genetic diversity. The ABGD method (Puillandre et al. 2012) was implemented online (http://wwwabi.snv.jussieu.fr/public/abgd/abgdweb.html), using the following settings: pmin = 0.001, pmax = 0.1, steps = 10, relative width gap (x-value) = 1, Nb bins = 20, and Simple Distance. Uncorrected *p*-distances were used instead of other distance metrics, as this is more suitable for closely related species (Machín-Sánchez et al. 2016).

The Generalized Mixed Yule Coalescent model (GMYC) infers species boundaries by combining a neutral coalescent model to a Yule stochastic model (Pons et al. 2006, Monaghan et al. 2009). This method applies single (GMYCs) or multiple (GMYCm) thresholds to delimit the transitions between intraspecific (coalescence) and interspecific (speciation) branching processes, fitting the GMYC model to a calibrated ultrametric tree. The ML implementation was performed on the GMYC Web Server (http://species.h-its.org/gmyc/) allowing single- and multiple-threshold models, using a Newick formatted tree as the input. An ultrametric consensus tree was constructed in BEAST v. 1.8.3 (Drummond et al. 2012) under a GTR + I + G model, a relaxed uncorrelated lognormal molecular clock, with a mean substitution rate fixed at 1 and estimated branch length with an extended Bayesian Skyline Plot coalescent as prior. MCMC chains were run for 10 million generations, with sampling every 1,000 generations after a burn-in period of 20%.

The Poisson Tree Process model (Zhang et al. 2013) uses gene tree branch lengths as number of mutations to model speciation and coalescent events by two independent Poisson process classes, and includes Maximum Likelihood (PTP) and Bayesian Inference (bPTP) implementations. PTP and bPTP analyses were performed on the bPTP Web Server (http://species.h-its.org/ptp/), employing the consensus tree previously produced in MrBayes as the input. The analyses were run applying 100,000 MCMC generations, with thinning to 100 and burn-in to 10%, and a five-digit random seed generator (12345).

RESULTS

Marker comparison. The success in amplification and sequencing reactions varied greatly among markers. Many of the available specimens of *Gibsmithia* were sequenced for UPA (45 sequences), while COI-5P had a lower success rate, with only 18 samples sequenced. Intermediate success rates were obtained with *rbc*L, with 20 samples sequenced only for the first or the second half of the gene and 24 complete sequences. Although the *rbc*L sequences with extensive missing data were not considered for

the phylogenetic analyses, they still served as "barcodes" (i.e., short diagnostic segments used to discriminate between the putative species; Hebert et al. 2003).

The three genetic markers presented different levels of sequence divergence among specimens belonging to the *Gibsmithia hawaiiensis* complex, COI-5P being the most diverse, UPA the most conserved, and *rbc*L with intermediate divergence (*p*-distance ranges of 0.0%–11.3%, 0.0%–2.8%, and 0.9%–6.3%, respectively; Fig. S1 in the Supporting Information). A large discontinuity in the genetic variation was observed for COI-5P (Fig. S2A in the Supporting Information), presenting a gap in the *p*-distance values between 0.5% and 1.6% (with the exception of specimens from Micronesia, where a specimen from Palau exhibited a divergence of 0.9%–1.0% from the specimens from Chuuk). Other gaps were also observed for COI-5P, but with much smaller intervals. A small discontinuity interval (0.7%–0.8%) was also found in *rbc*L, but not as clear as in COI-5P (Fig. S2B). A second interval was detected for *rbc*L (*p*-distance of 1.8%), reflecting a larger genetic discontinuity at another taxonomic rank. Discontinuity in UPA variation was not observed (Fig. S2C).

The same trend was observed for the parsimony-informative characters per site and the nucleotide diversity (π), with COI-5P having the highest values for the species complex (13.00% and 0.051), UPA the lowest (5.30% and 0.012), and *rbc*L intermediate (5.30% and 0.017). Nevertheless, the informative value and diversity of the larger gene (*rbc*L) increased considerably when sequences of higher taxonomic ranks were added to the dataset, resulting in similar values to COI-5P (Fig. S3 in the Supporting Information; Table S2 in the Supporting Information).

The high sequence divergence, the discontinuity in sequence variation and the general informativeness at the complex level corroborated the choice of the COI-5P dataset for single-marker SDMs.

Species delimitation methods. Species delimitation inferred from COI-5P detected different numbers of putative species belonging to the *Gibsmithia hawaiiensis* complex, depending on the method used. Using the standard 95% connection limit, SPN analysis resulted in eight taxa, while the 99% connection limit resulted in ten taxa. ABGD detected nine groups in the initial partition, either considering 0.001, 0.0017, or 0.0028 as prior intraspecific divergences. Both GMYCs and GMYCm recognized nine ML entities, with a confidence interval of 9–10 entities. PTP and bPTP also identified nine species, with highly supported partitions (minimum support of 0.706 for the Micronesian clade in both analyses). Although the number of putative species varied slightly among methods, the seven SDMs presented not only similar but also congruent results (i.e., all specimens belonged to equivalent lineages; Fig. 3). Combining several algorithmic methods for a more robust estimate of species boundaries (Kekkonen et al. 2015), nine putative species were recognized (Fig. 3). Nevertheless, further sampling is needed to resolve the phylogenetic relationship within the species from Micronesia (Chuuk, Pohnpei, and Palau) as well as between the two species from the Red Sea (one from Eilat and Hurghada, and another from Sharm El- Sheikh).

Phylogenetic analyses. Single-gene ML and BI analyses resulted in overall similar and congruent phylogenies, although the values of branch support varied for most nodes. COI-5P and *rbc*L presented comparable levels of node support (Figs. 3 and 4) while UPA exhibited very low support (Fig. S4 in the Supporting Information).

Sequences belonging to the *Gibsmithia hawaiiensis* complex clustered in a strongly supported group, comprising nine lineages (Fig. 3, A-I). Lineages A to E have been previously observed by Gabriel et al. (2016), and four more lineages (F–I) were newly recognized. The *G. hawaiiensis* complex, together with other *Gibsmithia* species, formed a well-supported clade, which is not strongly related to any other genera in the Dumontiaceae family.

The COI-5P phylogeny of the *Gibsmithia hawaiiensis* complex resulted in nine lineages distributed throughout four major groups: one group composed of lineages A + B + C + G + I; another group consisted of lineages E + H; whereas D and F formed single-lineage groups (Fig. 3). A total of 20 COI-5P haplotypes were detected, with an overall haplotype diversity of 0.963 (Table 1) and average pairwise distances between lineages from 1.8% to 9.2% (Table S3 in the Supporting Information).

The *rbc*L phylogeny resulted in two major groups, where one group was composed of lineages A + (B+I) + C + G, and one weakly supported group contained lineages D + E + F (Fig. 4). A total of seven *rbc*L lineages were detected, comprising 15 haplotypes (Table 1). Despite multiple attempts, a complete *rbc*L sequence could not be obtained for lineage H. However, a neighbor-joining analysis including partial *rbc*L sequences also detected lineage H (Fig. S5 in the Supporting Information). A notable difference was found for lineages B and I, which were delimited with COI-5P but could not be unambiguously distinguished with *rbc*L. This lineage (B + I) had an average *rbc*L *p*-distance of only 0.2% (Table S4 in the Supporting Information).

The UPA phylogeny showed much less variation than COI-5P and *rbc*L, but tree topology was mostly congruent between all markers. Although no strong support was observed for most UPA clades, all reported lineages could be detected except for lineages B, G, and I (Fig. S4). The haplotype network indicated that none of the fifteen UPA haplotypes are shared between individuals assigned to the nine mitochondrial lineages (Fig. 5), except between lineages B and I (sample DG206).

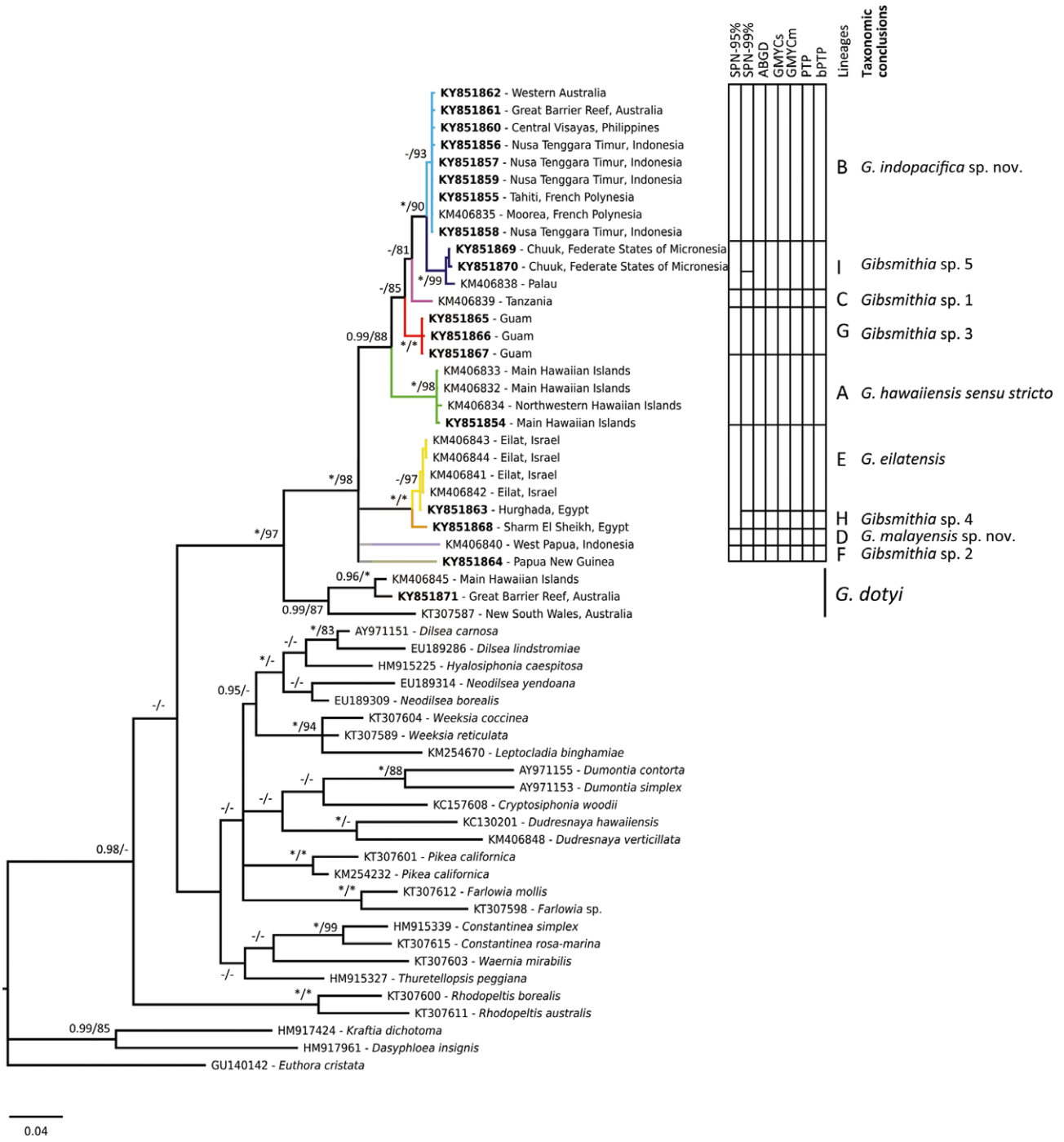

FIG. 3. Phylogenetic reconstruction of the *Gibsmithia hawaiiensis* complex as inferred from Bayesian Inference analysis of COI-5P sequences. Bayesian Inference posterior probabilities (PP) and Maximum Likelihood bootstrap (BS) presented as "PP/BS" near branches. * represent PP of 1.0 and BS of 100%. SPN-95%, statistical parsimony network with 95% connection limit; SPN-99%, statistical parsimony network with 99% connection limit; ABGD, automated barcode gap discovery; GMYCs, generalized mixed Yule-coalescent model under the single threshold method; GMYCm, generalized Mixed Yule-coalescent model under the multiple threshold method; PTP, Poisson tree processes; and bPTP, Bayesian implementation of the Poisson Tree Process. Newly generated sequences are presented in bold. Letters beside clades represent the different lineages observed (A–I). The other ingroup taxa are members of the Dumontiaceae, the outgroup taxon is a member of the Kallymeniaceae. [Color figure can be viewed at wileyonlinelibrary.com]

Lineages with shallow mtDNA differentiation had little separation in UPA (Table S5 in the Supporting Information), with the group comprised of lineages A, B, C, G, and I presenting very similar haplotypes. The average UPA *p*-distance among these lineages was 0.3%.

Morphology and taxonomy. Some phenotypic variation was observed in the general external habit,

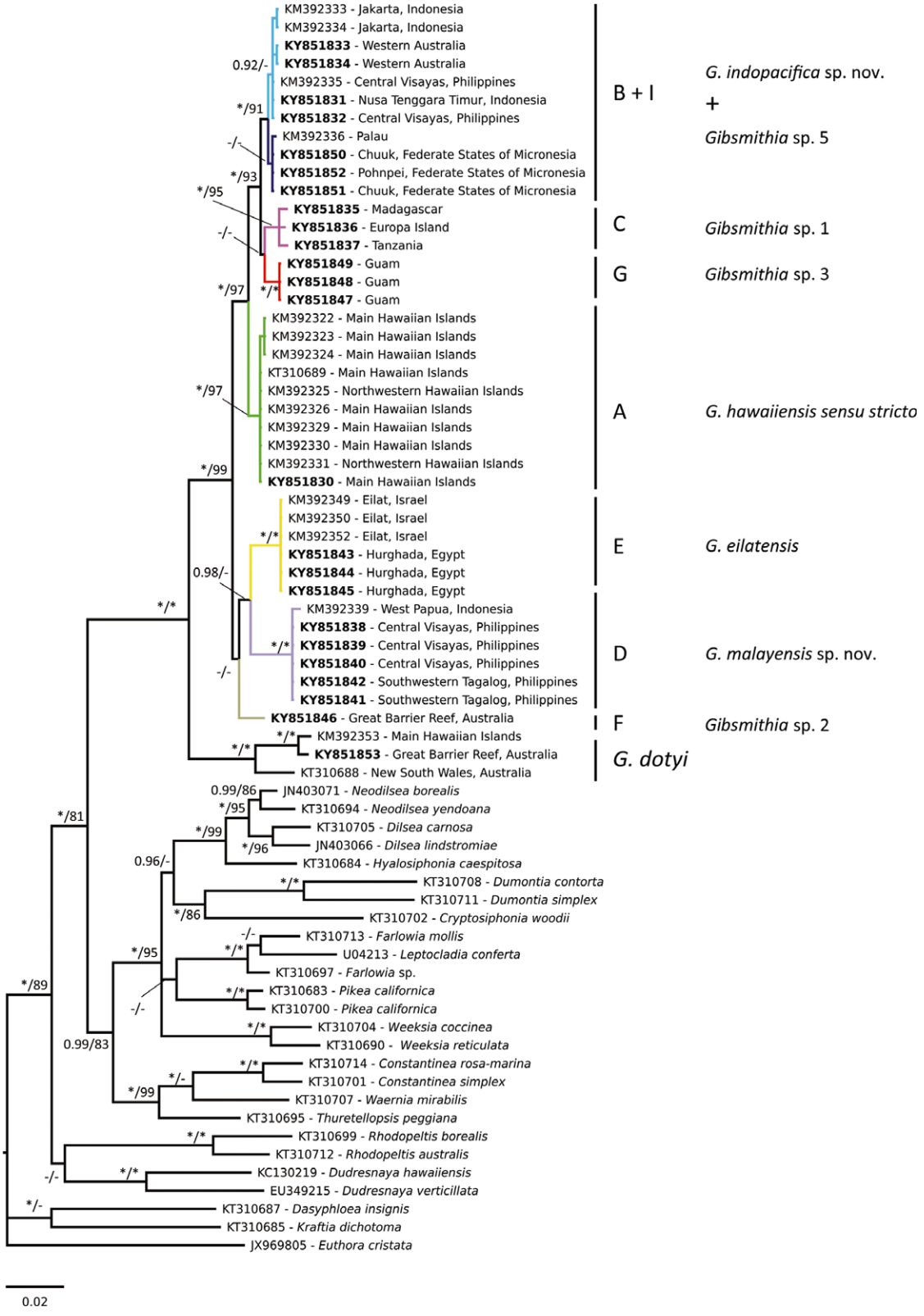

FIG. 4. Phylogenetic reconstructions of the *Gibsmithia hawaiiensis* complex as inferred from Bayesian Inference analysis of *rbc*L sequences. Bayesian Inference posterior probabilities (PP) and Maximum Likelihood bootstrap (BS) presented as "PP/BS" near branches. * represent PP of 1.0 and BS of 100%. Newly generated sequences are presented in bold. Letters A–I represent the different mitochondrial lineages (see Fig. 3). The other ingroup taxa are members of the Dumontiaceae, the outgroup taxon is a member of the Kallymeniaceae. [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 1. Diversity measures for lineages of *Gibsmithia hawaiiensis* complex. Sample size (N), number of haplotypes (h), haplotype diversity (Hd), and nucleotide diversity (π).

	N	h	Hd	π
COI-5P				
A	4	3	0.833	0.00261
B	9	5	0.722	0.00155
C	1	1	0.000	0.00000
D	1	1	0.000	0.00000
E	5	3	0.800	0.00174
F	1	1	0.000	0.00000
G	3	2	0.667	0.00116
H	1	1	0.000	0.00000
I	3	3	1.000	0.00696
Overall ingroup	28	20	0.963	0.05117
*rbc*L				
A	10	2	0.467	0.00044
B+I	11	5	0.855	0.00176
C	3	3	1.000	0.00506
D	6	2	0.333	1.00000
E	6	1	0.000	0.00000
F	1	1	0.000	0.00000
G	3	1	0.000	0.00000
H	–	–	–	–
Overall ingroup	40	15	0.919	0.01684
UPA				
A	10	1	0.000	0.00000
B+G+I	20	6	0.726	0.00289
C	5	2	0.400	0.00125
D	18	2	0.294	0.00092
E	9	1	0.000	0.00000
F	3	2	0.667	0.00208
H	4	1	0.000	0.00000
Overall ingroup	69	15	0.892	0.01219

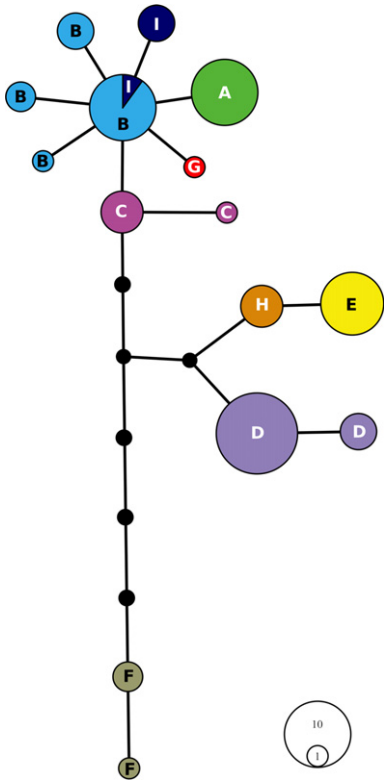

FIG. 5. Median-joining network of UPA haplotypes of the *Gibsmithia hawaiiensis* complex. Circle sizes correspond to the numbers of individuals sharing the same haplotype. Letters A–I represent the different mitochondrial lineages (see Fig. 3). [Color figure can be viewed at wileyonlinelibrary.com]

such as stalk length and branching, thallus color, length, and branching, as well as the density of "hair-like" filaments extending beyond the thallus' surface (Fig. 1). Nevertheless, the variation in these characteristics was not congruent with the genetic variation between genetic lineages within the *Gibsmithia hawaiiensis* complex.

Specimens belonging to lineages C, F, G, and I were only available as dried herbarium vouchers or preserved in silica gel, and could not be adequately rehydrated for histological studies due to the extremely gelatinous nature of the thalli.

Anatomical examination revealed morphological differences in the vegetative and reproductive structures (e.g., the origin of the percurrent filaments or the number of cells in the carpogonial branches). Although many specimens were not fertile, reproductive characters were observed in specimens belonging to lineages B and D, including gametophytic (male and female), carposporophytic and tetrasporophytic structures (Table 2). Samples belonging to lineage H were also investigated, but no fertile gametophytes were found.

Concordance between phylogenetic inferences, species delimitation models (SDMs), and morphological observations were combined to assess the plausibility of each putative species. This approach allowed the description of lineages B and D as *Gibsmithia indopacifica* sp. nov. and *Gibsmithia malayensis* sp. nov., respectively. In addition to the specimens sequenced in the present study, samples analyzed by Draisma et al. (2012) using DNA sequences of the intergenic spacer between the cytochrome oxidase subunits 2 and 3 (cox2-3 marker) were used for the new species descriptions. In their study, Draisma and colleagues recognized three undescribed species of *Gibsmithia*, with their *Gibsmithia* sp. 2 corresponding to our lineage B, and their *Gibsmithia* sp. 1 to our lineage D.

Gibsmithia malayensis D.Gabriel, Draisma and Fredericq sp. nov. (Figs. 1, D and E; 6–9, Fig. S6 in the Supporting Information)

Diagnosis: Medullary filaments cohesive in unbranched portions of the thallus, with cells becoming wider toward branched lobes (21–356 μm × 5–7.5 μm). Cortical branches alternate, sometimes opposite growing from larger percurrent filaments, branching pseudodichotomous, occasionally bearing rhizoidal filaments growing toward the thallus base. Cortical cells rectilinear, decreasing in size toward the surface (5–91 μm × 2.5–11 μm). Auxiliary cell branch of 12-(17)-26 cells. Carposporangia 9-(10)-

TABLE 2. Comparison of morphological features among species of *Gibsmithia hawaiiensis* complex. Characters based on [1]Doty 1963, [2]Gabriel et al. 2016 and [3]the present study.

Species	*G. hawaiiensis*[1]	*G. eilatensis*[2]	*G. malayensis* sp. nov.[3]	*G. indopacifica* sp. nov.[3]
Cortex	Main percurrent filaments oppositely or radially bearing pseudodichotomously branched cortical filaments	Main percurrent filaments alternately bearing unbranched or sparingly alternately branched cortical filaments	Main percurrent filaments alternately sometimes oppositely bearing pseudodichotomous branched cortical filaments or occasional rhizoidal filaments	Main percurrent filaments laterally and oppositely bearing pseudodichotomously branched cortical filaments or slender rhizoidal filaments
Seirospores	In unbranched chains	In unbranched chains with occasional branchlets	Not reported	In branched chains
Sexuality	Not reported	Dioecious	Monoecious	Monoecious
Carpogonial branch	4 modified spherical cells, with subhypogynous cell sometimes smaller, and carpogonium borne terminally and excentrically at an angle of 45°	5–9 modified spherical cells, with laterally enlarged hypogynous cell, very small subhypogynous cell, and carpogonium borne terminally and excentrically	6–8 modified cells, subhypogynous smaller. Carpogonium borne at the corner of hypogenous cell	5–7 differentiated. Carpogonium borne laterally on slightly oval hypogynous cell with straight trichogyne. Subhypogynous cell the smallest in the branch
Connecting filament development	Not reported	Connecting filaments septate	Connecting filament cut off from the bridge formed by the fusion of carpogonium and the 3 cells below it. Connecting filament become septate only before and after contacting with auxiliary cells	Septate connecting filaments cut off from bridge-like structure formed with the partial fusion of the caropogonium and the 2 cells bellow it.
Auxiliary cell branch	4 modified spherical cells	3–7 modified spherical cells with auxiliary cell smaller	5–6 modified cells rounder	3–5 modified semispherical cells
Cystocarp development	Gonimoblast formed from the junction of a connecting filament with the auxiliary cell	Gonimoblast formed from the short segment of a connecting filament fused with the auxiliary cell	Gonimoblast initial cut off from lateral bulge formed after partial fusion of the auxiliary cell with the outgoing connecting filament	Gonimoblast originated from connecting filament after fusion with auxiliary cell
Spermatangia	Not reported	Spermatangia borne radially on 3(5)6 terminal cortical cells. Spermatangial heads borne in a similar position of tetrasporangia	Spermatangia lateral or terminal on cortical branches, borne on 4–8 apical cortical cells	Spermatangia sessile, adaxial on cortical filaments, with spermatanmgial mother cells borne radially on 4–6 terminal cortical cells
Tetrasporangia	Tetrasporangia are mostly decussate, rarely tetrahedral or cruciate, borne laterally from inner cortical cells on a 2–3-celled pedicel	Tetrasporangia are mostly decussate, rarely cruciate, borne unilaterally or terminally on short laterals on inner cortical branches	Tetrasporangia decussate, rarely cruciate, borne in pairs on single-celled pedicel. Tetrasporangia spiral around the cortical filaments	Tetrasporangia borne in clusters, 2–4 per 1–2-celled lateral alternate branchlets, sometimes spiral around cortical filaments
Confirmed distribution	Hawaii: Main and Northwestern Hawaiian Islands	Red Sea: Eilat (Israel), Hurghada (Egypt), Farasan Banks (Saudi Arabia)	Central Indo-Pacific: West Papua and North Moluccas (Indonesia); Sabah (Malaysia); Camiguin, Calamian, Bohol, Cebu and Siquijor (Philippines)	Central and Eastern Indo-Pacific: Jakarta and Nusa Tenggara Timur (Indonesia); Johor and Sabah (Malaysia), Siquijor, Cebu and Sorsogon provinces (Philippines); Western Australia and Queensland (Australia); Moorea and Tahiti (French Polynesia)

12.5 μm × 7.5-(10)-12.5 μm. Spermatangial heads, 26–69 μm × 15–35 μm, with spermatia borne on 4–8 apical cortical cells. Tetrasporangia 17–23 μm × 14.5–21 μm, decussate, rarely cruciate, borne in pairs on single-celled pedicel.

Holotype: Deposited in L: SGAD0712172 (monoecious gametophyte), "Maya's Mimpi," Raja Ampat, Indonesia, 00°30′27″ S, 130°39′55″ E, November 25, 2007, 10 m depth, collected by S.G.A. Draisma.

Additional specimens examined: Deposited in KLU: SGAD1012018 (tetrasporophyte), Sabah, Malaysia. Deposited in L: SGAD0712139 (sterile), SGAD 0712172 (monoecious gametophyte), SGAD0712653 (monoecious gametophyte), SGAD0712751 (female gametophyte) – Raja Ampat, Indonesia; SGAD140 3028 (female gametophyte), SGAD1403097 (male gametophyte), SGAD1403107 (female gametophyte) Calamian – Philippines; SGAD1312058 (monoecious gametophyte), SGAD1312071 (female gametophyte), SGAD1312088 (monoecious gametophyte), SGAD13 12100 (male gametophyte), Cebu – Philippines; SGAD1401033 (female gametophyte) Siquijor – Philippines; SGAD1404024 (female gametophyte), SGAD1404052 (sterile) Camiguin – Philippines.

Etymology: The species is named for the general collection area, the Malay Archipelago.

Representative sequences deposited in GenBank: KM406840 (COI-5P), KM392339 (rbcL) and KM392308 (UPA).

Confirmed distribution: Indonesia: Raja Ampat (West Papua province), Ternate (North Moluccas); Malaysia: East Semporna and Sandakan (Sabah); Philippines: Camiguin province (Northern Mindanao Region), Calamian province (Southwestern Tagalog Region), Bohol, Cebu and Siquijor provinces (Central Visayas Region).

Ecology: Subtidal to 25 m depth. When present, few specimens (usually up to three) were found during a single dive, growing on live coral. Specimens were collected year round except for February, August and September (although its occurrence cannot be excluded from these periods since no sampling effort was made).

Habit and vegetative morphology: An unbranched cartilaginous stalk bears 7–14 light-to dark-pink furry gelatinous variously sized roundish lobes (Fig. 1, D and E) extending from a compressed, unbranched lower thallus portion (Fig. 6, Fig. S6, A–E). Cohesive medullary filaments are colorless, distributed in the unbranched portion of the thallus, with cells becoming wider toward branched lobes (5–7.5 μm × 21–356 μm; Fig. 7A). Small cells borne laterally and alternately on main medullary filaments each often subtend three percurrent cortical filaments. Assimilatory filaments consist of main percurrent filaments that laterally bear unbranched, straight, smaller celled cortical filaments (Fig. 7A) and cut off thin, narrow rhizoidal filaments growing thallus inward and basipetally (Fig. 7A) where they intertwine with medullary filaments. Lower percurrent filaments may cut off short rectangular segments laterally, with each segment dividing in a pseudodichotomous fashion forming straight cortical filaments (Fig. 7B). Cortical filaments are typically cut off alternately and occasionally opposite from larger percurrent filaments and are 17–32 cells long. Cortical cells are rectilinear, decreasing in size toward the surface (5–91.3 μm × 2.5–11.3 μm). Hair-like structures (1.3 μm × 80–121.3 μm) occasionally project from outer cortical cells. Seirospores are present in long branched chains (Fig. 7C). Evidence of the release of seirospores (as reported by Karam-Kerimian 1976) was not observed.

Reproductive morphology: Reproductive structures were not found on small blades. Plants are monoecious, with isomorphic tetrasporophytes. Carpogonial branches are straight (Fig. 7D), positioned in the central portion of cortical filaments, 18–29 cells below the surface. They consist of nearly similar-sized cells besides a slightly smaller, roundish subhypogynous cell (Fig. 7D). Of the 6–8 cells forming the carpogonial branch, only the upper 5–6 cells stain darkly with aniline blue. The carpogonium is positioned laterally from the hypogynous cell and bears a straight trichogyne at a 45° angle from the carpogonial branch (Fig. 7D). Following presumed fertilization, the lower portion of the fertilized carpogonium extends toward the base of the branch, partly fusing with the hypogynous, the subhypogynous cell, and the cell below it resulting in a narrow bridge from which connecting filaments primordia are issued (Fig. 7E). Auxiliary cell branches are composed of 12-(17)-26 cells (Fig. 7F), the upper 5–16 cells remaining vegetative, the 4–5 cells below the auxiliary cell become round and darkly staining while the basalmost 2–5 cells cut off straight or curved unbranched lateral filaments. Four to five cells below the tip of the auxiliary filament become roundish and darkly staining with aniline blue, except for the auxiliary cell that becomes hyaline, observable even in auxiliary cell filaments that are not diploidized (Fig. 8A). Auxiliary cell branches that are not diploidized are converted into vegetative filaments that grow outward (Fig. 7F). A dome-shaped gonimoblast initial is cut off (Fig. 8B) from the lateral bulge formed after the partial fusion of the auxiliary cell with the incoming connecting filament that continues growing toward neighboring auxiliary cells (Fig. 8B at left, 8C). Several small gonimoblast cells are typically cut off from the auxiliary cell bulge subsequent to the gonimoblast initial (Fig. 8C) and mature simultaneously within a globular carposporophyte reaching 80–115 μm × 105–130 μm. All cells become carposporangia reaching sizes of 8.8-(10)-12.5 μm × 7.5-(10)-12.5 μm. Lateral filaments cut off from the lower cells of the auxiliary cell branch continue to grow as the gonimoblasts develop, forming a loose, rudimentary involucre (Fig. 8D) around the maturing carposporophytes (Fig. 9A) spread throughout the thallus (Fig. 9B).

Spermatangia are cut off on lateral branchlets or terminally on cortical filaments (Fig. 9C), with spermatangial parent cells (1.5–2 μm diameter) radially borne on the last 4–8 apical cells (Fig. 9D), forming a corn cob-like spermatangial head (26.3–68.8 μm × 15–35 μm). If male and female structures are present on a same thallus, the former are typically formed close to the surface, and the latter 10 or more cells below the surface.

Tetrasporangia are typically borne 8–14 cells below the surface on cortical filaments (Fig. 9E), occasionally terminally on the cortical filaments. Tetrasporangial initials are cut off in two ways: either as a protrusion cut off obliquely from any upper part of a cortical cell resulting in a sessile tetrasporangium (Fig. 9E), or as the terminal cell of a two-celled lateral resulting in a pedicellate tetrasporangium (Fig. 9, E and F). Tetrasporangia spiral around the cortical filaments usually in pairs, with one more mature than the other (Fig. 9E). Tetrasporangia (17.2–22.9 μm × 14.5–21.1 μm) are mostly decussate, rarely perfectly cruciate.

Gibsmithia indopacifica D.Gabriel, Draisma and Fredericq sp. nov. (Figs. 1, G and H; 10–14, Fig. S7 in the Supporting Information)

Diagnosis: Medullary filaments loosely aggregated throughout the thallus, with uppermost cells (92–470 μm × 7–25 μm) transitioning into cortical cells thallus outward. Small round cells cut off laterally and oppositely on main percurrent filaments bearing cortical filaments and slender rhizoidal filaments; cortical filaments straight or pseudodichotomous, up to 26–38 cells long; rectilinear cortical cells decreasing in size toward the surface (5–66 μm × 4–11 μm). Terminal cortical hair-like structures rare, small (8–10 μm × 2–3 μm). Auxiliary cell branches of 13–22 cells. Spermatangial heads sessile, 29–45 μm × 14–18.5 μm. Spermatia 3–5 μm × 2.5–5 μm, borne radially on 4–6 terminal cortical cells. Tetrasporangia 20–25 μm × 15–19 μm, 2–4 per 1–2 celled lateral alternate branchlets.

Holotype: Deposited in L: SGAD1401011 (monoecious gametophyte), Paliton Wall (off Paliton beach), Siquijor, Philippines, 09°09′ 36″ N, 123°28′ 45″ E, January 6, 2014, 25 m depth, collected by S. Draisma.

Additional specimens examined: Deposited in GENT: HV94 (tetrasporophyte) – Moorea, French Polynesia. Deposited in KLU: SGAD1205027 (tetrasporophyte), SGAD1205043 (male gametophyte); SGAD1205144 (female gametophyte) – Johor, Malaysia.; SGAD1209261 (tetrasporophyte); SGAD1209360 (male gametophyte) – Sabah, Malaysia.

Etymology: The specific epithet refers to the general distribution of the species in the Indo-Pacific basin.

Representative sequences deposited in GenBank: KY851860 (COI-5P), KY851832 (rbcL) and KY851790 (UPA).

Confirmed distribution: Indonesia: Kepulauan Seribu (Jakarta province), Pantar Strait (Nusa Tenggara Timur province); Malaysia: Johor and Sabah, Philippines: Siquijor and Cebu provinces (Central Visayas region), and Sorsogon province (Bicol region); Australia: Ningaloo Reef (Western Australia), Great Barrier Reef (Queensland); French Polynesia: Moorea and Tahiti.

Ecology: Plants isolated or in groups of up to 4 individuals, sometimes joined at the base of the stipe, subtidal to 25 m depth, growing in cavities of coral reefs. Specimens were collected from February to October but collections were made opportunistically instead of continuously throughout the year.

Habit and vegetative morphology: Cartilaginous stalks are of variable size, from a few millimeters to 2 cm tall, bear 7–15 furry, closely appressed, elliptical to suborbiculate gelatinous lobes (Figs. 1, G and H; 10, Fig. S7, A–C), often spathulate (Fig. S7, D–F) with the upper parts of a lobe occasionally dividing into a round bifurcation. The color of the thalli ranges from whitish-to dark pink or red (Fig. 1, G and H). The branching pattern of the lobes differs considerably among individual samples depending on the site of attachment and depth (probably a response to different exposure to light, current, and sand abrasion). Medullary filaments are loosely aggregated throughout the blade, with cells enlarging when transitioning into cortical cells (92–470 μm × 7–25 μm). Small round cells are cut off laterally and oppositely on main percurrent filaments bearing narrow rhizoidal filaments (Fig. 11A), cortical filaments (Fig. 11B) and auxiliary cell filaments (Fig. 11C) commonly originate in opposite pairs. Cortical filaments are unbranched or pseudodichotomous, up to 26–38 cells long, and consist of rectilinear cortical cells (5–66 μm × 4–11 μm) that gradually decrease in size toward the surface (Fig. 11C). Terminal hair-like structures are rare on outer cortical cells and remain small when present, 8–10 μm × 2–3 μm (Fig. 11B).

Reproductive morphology: Plants are monoecious, with isomorphic tetrasporophytes. Straight carpogonial branches grow laterally on lower cells of cortical filaments (Fig. 11, C–E), 16–22 cells below the surface. Their position is homologous to those of cortical filaments and auxiliary cell branches. Carpogonial branches are composed of (7-8)-12 cells, with the more distal 5–7 cells staining darkly (Fig. 11, D and E), the four basal-most cells occasionally with lateral branchlets. Immature trichogynes are darkly staining and cytoplasm-rich (Fig. 11D). The carpogonium is conical, borne laterally on oval or slightly oval hypogynous cell, and extends in a straight trichogyne growing straight or obliquely toward the thallus surface. Concomitant with the expansion in size of the hypogynous cell, the carpogonium frequently rotates from a lateral position (Fig. 11D) to a terminal position at 45° angle to the abaxial side of the oval hypogynous cell (Fig. 11E). The subhypogynous cell is the smallest cell (Fig. 11E) in the carpogonial branch. Auxiliary

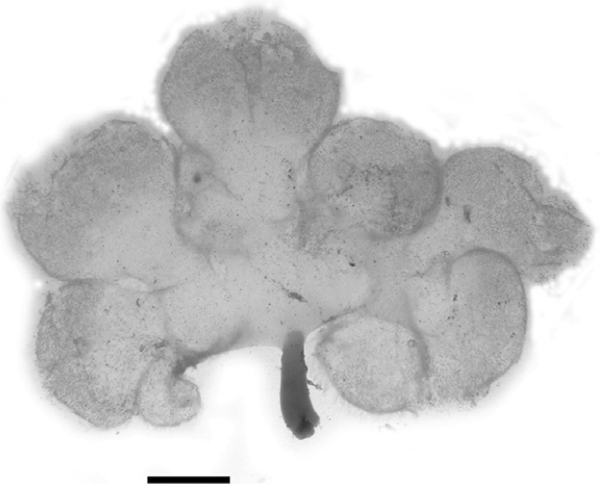

FIG. 6. Habit of *Gibsmithia malayensis* sp. nov. Formalin-preserved. Holotype, monoecious gametophyte, SGAD0712172, Raja Ampat, Indonesia, scale bars 1.0 cm.

cell branches are sometimes in close proximity to carpogonial branches. Young auxiliary cell branches are transformed cortical filaments from which they are easily distinguished by the presence of intercalary darkly staining cells that become roundish (Fig. 11F). The 3–6 lowermost cells of an auxiliary cell branch do not transform cytologically and frequently cut off small, narrow cells unilaterally (Fig. 11F) that may continue to divide. Auxiliary cell branches are composed of 13–22 cells, with 3–5 cells modified. The upper 5–8 more distal auxiliary branch cells do not become morphologically or cytologically transformed (Fig. 11F). After presumed fertilization, the carpogonium extends basally, with the narrow extension slightly contacting (Fig. 12A) the transformed hypogynous cell before continuing as a darkly staining connecting filament primordium. In some cases, the carpogonial extension becomes detached from the cell below the subhypogynous cell (Fig. 12A). Occasionally, the carpogonial extension slightly brushes against the hypogynous cell, continues its course below, partly fuses with the cell below the subhypogynous cell (Fig. 12B), septates and the lower extension fuses laterally with the cell below (Fig. 12C), forming a bridge-like structure issuing multiple connecting filaments. The small subhypogynous cell is not part of the carpogonial bridge (Fig. 12, A–F). The carpogonial bridge emits 2 (Fig. 12, D and E) to 3 (Fig. 12E) connecting filaments that are septated at their point of origin. The darkly staining carpogonial branch cells, with which the carpogonial extension partly fuses, progressively lose their cytoplasmic content (Fig. 12, E and F). Segmented connecting filaments grow toward auxiliary cells where diploidization occurs (Figs. 12G and 13A). After fusing with the auxiliary cell new outgoing connecting filaments are produced to reach other nearby auxiliary cells (Fig. 13, A and B). Connecting filaments from one fertilized carpogonial branch can contact at least five auxiliary cells. Gonimoblast initials are cut off from the portion of the connecting filament fused with auxiliary cell (Fig. 13B). No further stages of carposporophyte development were observed.

Spermatangial heads are 30–45 μm × 14–18.5 μm, sessile, distributed 4–13 cells below the surface and often adaxially inserted or terminal on a cortical filament (Fig. 13, C–E). Spermatangial heads develop from short, small lateral protrusions cut off obliquely from the upper part of an intercalary cortical filament (Fig. 13D). The resulting lateral branchlets (4–6 cells long) remain small and narrow and each of these tiny cells cut off small cells radially that become the narrow spermatangial parent cells (Fig. 13D) which themselves continue to divide in a similar fashion. Each of these spermatangial parent cells cuts off a spermatangium enclosing a spermatium (3–5 μm × 2.5–5 μm), forming a corn cob-like structure. If male and female structures are present in the same thallus, the former appears only after the presumable diploidization of the auxiliary cells.

Tetrasporangia are borne unilaterally on alternately, sparingly branched cortical filaments (Fig. 14, A–C). Tetrasporangial initials form laterally on 1–2 celled laterals cut off from cortical filaments 10–19 cells below the surface. These 1–2 pedicellate cells each typically continue to cut off 1–2 additional tetrasporangial initials unilaterally from their upper parts by oblique divisions (Fig. 14, D and E). The first set of mature, cruciately divided tetrasporangia, 20–25 μm × 15–19 μm (Fig. 14E), is followed by a second immature set.

Species distributions. Gibsmithia hawaiiensis sensu stricto (lineage A) is apparently restricted and hence endemic to the Hawaiian Islands (Fig. 2A), presenting very low genetic variation (Tables S3–S5). *Gibsmithia eilatensis* (lineage E) is apparently confined to the Red Sea (Fig. 2, A and B), but besides its type locality (Eilat, Israel), new records are reported from Hurghada (Egypt) and Farasan Banks (Saudi Arabia). Additional sampling from nearby regions is necessary to confirm the endemicity of *G. eilatensis*. The closely related lineage H is newly reported from Dahab and Sharm El-Sheik (Egypt), overlapping in distribution with its sister lineage (Fig. 2, A and B), *G. eilatensis*. *Gibsmithia indopacifica* (lineage B) presents the broadest distribution range, with records extending from Indonesia to French Polynesia (Fig. 2A), having sympatric populations with *G. malayensis* (lineage D), which is restricted to the Coral Triangle (Fig. 2, A and C). Lineage I, found in Micronesia, is closely related to *G. indopacifica*. Furthermore, the haplotype from Palau seems more closely related to *G. indopacifica* than the haplotype from Chuuk and Pohnpei, which reflects the increasing geographic distance between the haplotypes

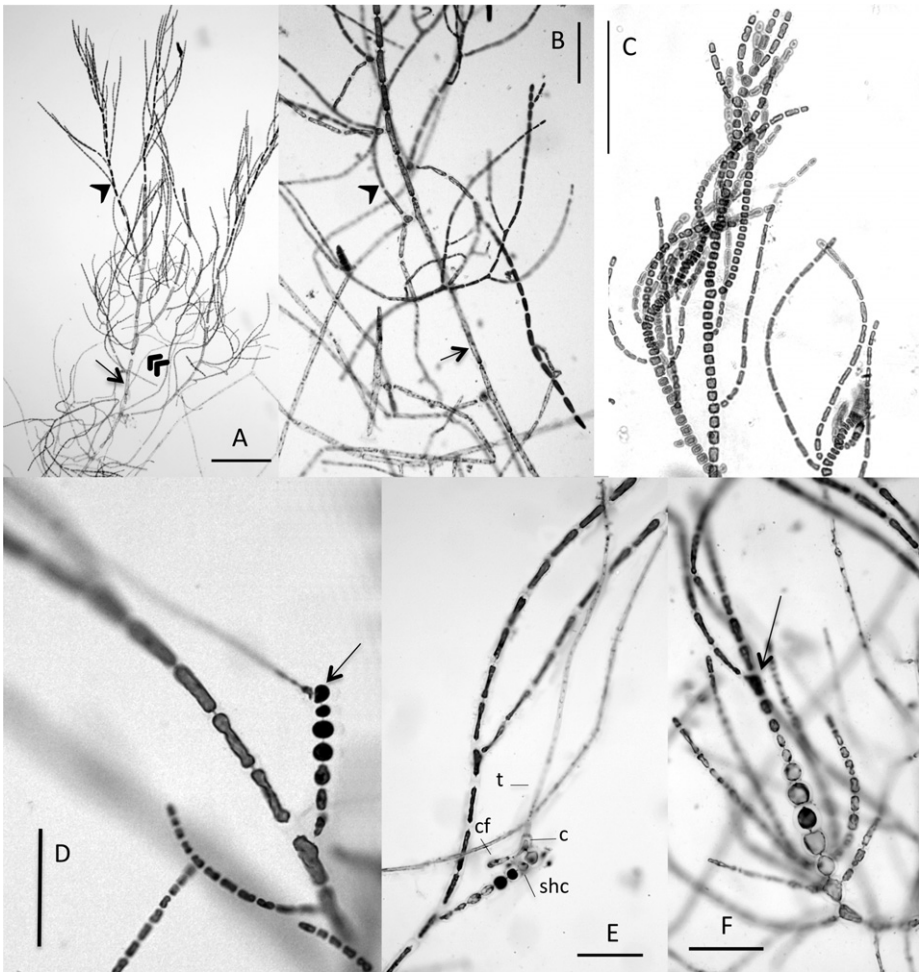

FIG. 7. *Gibsmithia malayensis* sp. nov. (A) Sparsely branched assimilatory filaments consisting of main percurrent filament (arrow), cortical filaments (arrowhead), and rhizoidal filaments (double arrowhead) growing basipetally. Slide SGAD0712751-1, scale bar 200 μm. (B) Percurrent filaments (arrow) with lateral segment (arrowhead) extending into branched cortical filaments. Slide SGAD0712139-1, scale bar 100 μm. (C) Seirospores in branched chains. Slide SGAD1312088-1, scale bar 100 μm. (D) Unfertilized carpogonial filament with roundish hypogynous cell (arrow). Slide SGAD0712751-1, scale bar 50 μm. (E) Connecting filament (cf) primordium extended from fertilized carpogonium (c) after its partly fusion with hypogynous cell and cell below the subhypogynous cell (shc), and persisting trichogyne (t). Slide SGAD0712172-2, scale bar 50 μm. (F) Non-functional auxiliary cell branch has converted into vegetative filament (arrow). Slide SGAD0712172-2, scale bar 50 μm.

(Fig. 2, A and C). Closely related to *G. indopacifica* and lineage I is the newly reported lineage G from Guam. Lineage F, the most genetically divergent lineage in the complex, is newly reported from the Central Indo-Pacific, occurring sympatrically with *G. indopacifica* in the Great Barrier Reef (Fig. 2, A and C). The previously reported lineage C is genetically diverse and appears to be restricted to the Western Indian Ocean (Fig. 2A).

DISCUSSION

Using multiple lines of evidence, the present study demonstrates that the genetic diversity within the *Gibsmithia hawaiiensis* complex is even greater than previously reported by Gabriel et al. (2016). Species delimitation inferred from COI-5P sequences and phylogenetic reconstructions based on *rbc*L, UPA and COI-5P showed congruent results, raising the number of lineages within the complex from five to nine. Besides the previously described *G. hawaiiensis* sensu stricto (lineage A) and *G. eilatensis* (lineage E), the newly described *G. indopacifica* (lineage B) and *G. malayensis* (lineage D) have doubled the number of formally described species in the complex to four. Furthermore, lineages C, F, G, H, and I represent putative species awaiting additional studies to confirm their taxonomic status and enable their eventual description.

Although habit characteristics did not differ between members of the *Gibsmithia hawaiiensis* complex, some species can be separated from each other at a microscopic level, through a combination of differences in their vegetative and reproductive structures (Table 2). For example, cortical filaments in *G. eilatensis* are rarely cut off opposite each other from the main percurrent filaments and are usually unbranched or sparingly alternately branched, unlike those of *G. hawaiiensis* sensu stricto. Percurrent filaments have a simple origin in *G. hawaiiensis* and *G. eilatensis*, growing directly from main medullary filaments. In contrast, percurrent filaments in *G. malayensis* and *G. indopacifica* grow from small lateral cells borne on main medullary filaments, with these small lateral cells having an alternate arrangement in the former and an opposite organization in the latter. Carpogonial branches bear a very small subhypogynous cell in *Gibsmithia eilatensis*, while all

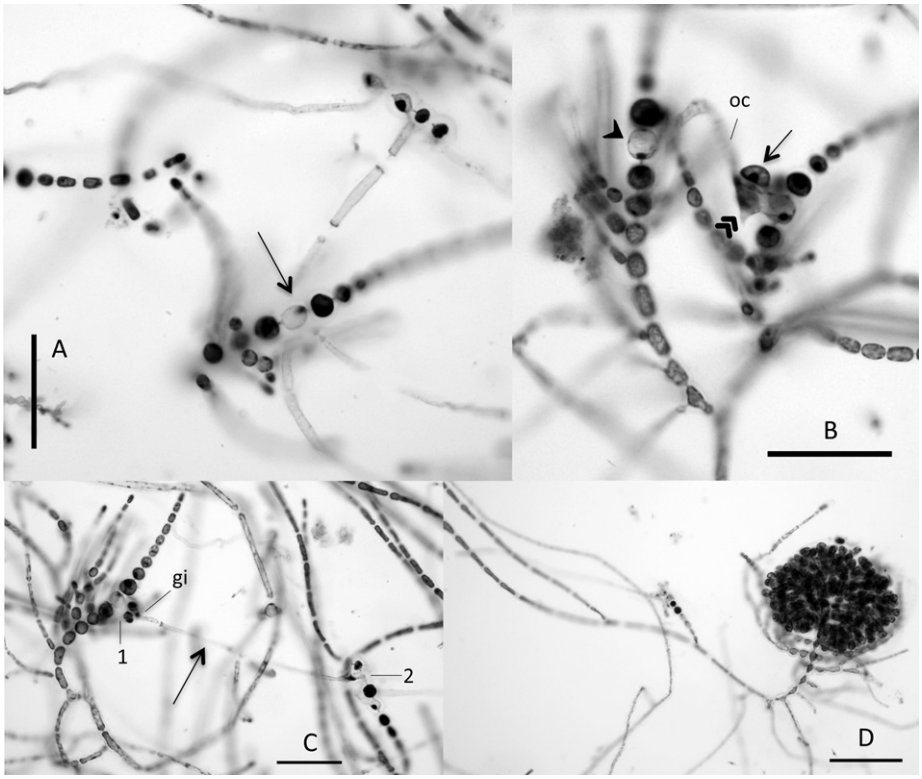

FIG. 8. *Gibsmithia malayensis* sp. nov. (A) Non-diploidized auxiliary cell branch (arrow). Slide SGAD0712653-1, scale bar 50 μm. (B–D) Post-fertilization stages. (B) Auxiliary cell branch (at right) with gonimoblast initial (arrow) cut off from fusion product of outgoing connecting filament (oc) with auxiliary cell, and diploidization attempt of other auxiliary cell (arrowhead) in its vicinity. Slide SGAD0712653-2, scale bar 50 μm. (C) Early post-fertilization showing septate connecting filament (arrow) connected to two auxiliary cells (1 and 2) with 1 bearing gonimoblast initials (gi). Slide SGAD0712653-2, scale bar 50 μm. (D) Mature carposporophytes. Slide SGAD0712172-2, scale bar 100 μm.

cells of the carpogonial branch are similar in size and shape in other species of the complex. In comparison to *G. hawaiiensis* and *G. eilatensis*, cortical cells appear to be more squarish and narrower in *G. malayensis* and *G. indopacifica*. The only *Gibsmithia* species known to be monoecious are *G. malayensis*, with mature female and male structures occurring concomitantly in the same individuals, and *G. indopacifica*, with male structures maturing in the same individual as female structures only after the auxiliary cells have been diploidized. This sequential maturation could presumably avoid self-fertilization in *G. indopacifica*. Spermatangial heads in *G. malayensis* are more elongate (covering up to eight cortical cells) than those of *G. indopacifica* and *G. eilatensis* (usually with five cortical cells bearing spermatangia). Since spermatangia of *G. indopacifica* were observed when female reproductive structures on a same monoecious thallus were already formed, this species appears to be protogynous an unusual condition in the family. Tetrasporangia are mostly borne on inner cells of cortical filaments in all species of the complex, but may occasionally be terminal in *G. eilatensis* and *G. malayensis*. Tetrasporangia are borne in pairs directly from cortical filaments in *G. malayensis*, and in short lateral branchlets in other members of the complex. Tetrasporangia in *G. eilatensis* and *G. malayensis* are mostly decussate and rarely cruciate as sometimes also observed in *G. hawaiiensis* and *G. indopacifica*. Spermatangia and initial post-fertilization events are still unreported for the generitype since they were not observed in the specimens of *G. hawaiiensis* from the Hawaiian Islands described by Doty (1963) and Kraft (1986) (specimens MELU K1277 and MELU K1284). Other descriptions of *G. hawaiiensis* are based on specimens from other locations (e.g., Karam-Kerimian 1976 for New Caledonian and Tahitian samples; Kraft 1986 for Australian (MELU K15521, MELU K15605, MELU K15675, MELU K15755, MELU K15761, MELU K16181, MELU 23004–MELU 23010, MELU 23019, MELU 23021), Philippine (MELU K869, MELU K878, MELU K957) and Papua New Guinean (LG 5825) samples) and therefore should be used with care.

The high genetic divergences within *Gibsmithia indopacifica* and *G. malayensis* probably result from the wide geographic distribution currently observed for the populations of both species (Draisma et al. 2012). This high diversity may also represent genetic admixture or mixed ancestry, with populations from different sources mixing through time during

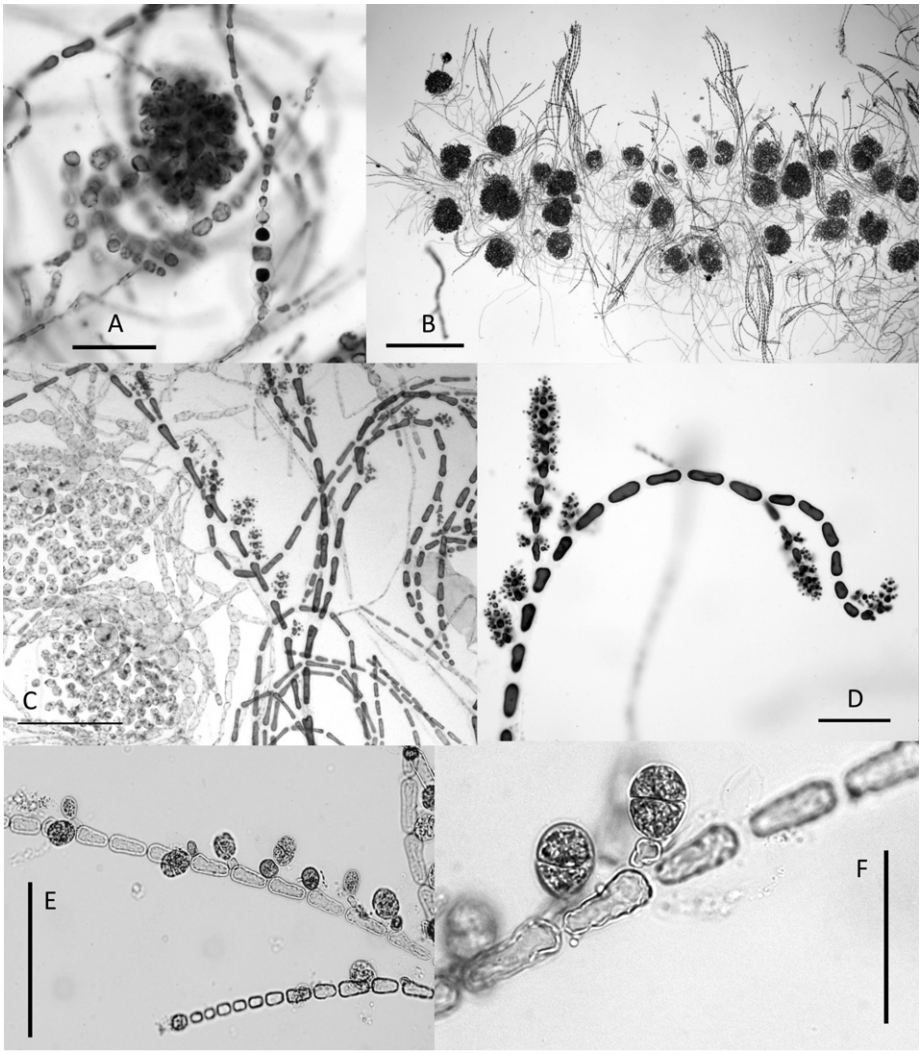

FIG. 9. *Gibsmithia malayensis* sp. nov. Post-fertilization, male and tetrasporangial stages. (A, B) Mature carposporophytes. (A) Slide SGAD0712172-2, scale bar 50 μm. (B) Slide SGAD0712172-2, scale bar 200 μm. (C, D) Spermatangial phases. (C) Young spermatangial clusters in monoecious specimen (note carposporophytes at left). Slide SGAD1312058-1, scale bar 100 μm. (D) Young and mature spermatangial clusters in mostly terminal position on cortical filaments. Slide SGAD0712653-1, scale bar 50 μm. (E, F) Pedicellate tetrasporangial initials and tetrasporangia. (E) Slide SGAD1012018-1, scale bar 100 μm. (F) Slide SGAD1012018-1, scale bar 50 μm.

glaciations (Carpenter et al. 2011), which may have prevented further speciation by homogenizing populations while maintaining heterogeneous haplotypes, especially in Southeast Asia. On the other hand, *Gibsmithia hawaiiensis* and *G. eilatensis* represent very conserved clades, probably resulting from bottlenecks due to founder effects (Paulay and Meyer 2002) and dispersal limitations due to geographic isolation (Rossetto et al. 2008), resulting in low genetic diversity and homogeneous haplotypes. Both events are reported by Wörheide et al. (2008) for reef sponges with cryptic diversity, but further studies at the population level are necessary to test these hypotheses in the *G. hawaiiensis* complex.

Members of the *Gibsmithia hawaiiensis* complex are found growing in small groups (Huisman et al. 2007; S. G. A. Draisma and C. F. D. Gurgel, pers. obs.), occasionally on reef flats, but usually among branches of corals (Magruder and Hunt 1979), protruding from small undercuts and crevices, or at the entrance of shallow holes (Littler and Littler 2003), in such a way that only the upper branches are visible (Huisman et al. 2007). Reproductive structures are found in patches along the tips of the gelatinous branches (Doty 1963). Thalli are believed to be seasonal and shed annually (Huisman et al. 2007), whereas the cartilaginous stalks of *Gibsmithia* appear to have annular growth rings, suggesting that the stalks are perennial (Huisman 2000). Gamete and spore dissemination could be optimized by their presence in deciduous, gelatinous branches, while the perennial, cartilaginous stalk may enhance the probability of thallus survival until the next reproductive season. Signs of herbivory were observed in some of the studied specimens (e.g., TS931 from the Red Sea, SGAD0911085 from Indonesia, HV94 from French Polynesia, TS348 from Hawaii) as previously reported by Kraft (1986) for Australian specimens.

Previously reported records of the *Gibsmithia hawaiiensis* complex are here confirmed for Papua New Guinea and the Great Barrier Reef (Kraft 1986), Western Australia (Huisman 2000), Guam (Lobban and Tsuda 2003), and Europa

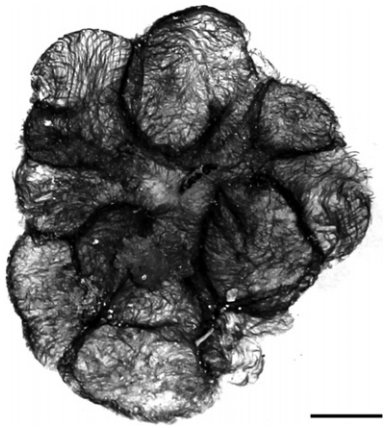

FIG. 10. Habit of *Gibsmithia indopacifica* sp. nov. Herbarium-pressed. Holotype, monoecious gametophyte, SGAD1401011, Siquijor, Philippines, scale bars 1.0 cm.

Island (Mattio et al. 2016). The geographic distribution of the species complex is better documented with new records from Dahab, Sharm El-Sheik and Hurghada in Egypt; Farasan Banks in Saudi Arabia; Nusa Tenggara Timur in Indonesia; Siquijor and Bohol (Central Visayas), Palawan and Calamian (Southwestern Tagalog), and Camiguin (Northern Mindanao) in the Philippines; and Chuuk and Pohnpei in the Federated States of Micronesia, indicating that the complex is more widespread in the Indo-Pacific than previously thought (Gabriel et al. 2016).

Species belonging to the *Gibsmithia hawaiiensis* complex differ from their congeners by the hairy appearance of the gelatinous lobes, which are due to cortical filaments that extend beyond the thallus surface (Gabriel et al. 2016). Those cortical filaments originate on cells of percurrent cortical axes (Kraft 1986), which are also exclusive to this species complex, since other *Gibsmithia* species present a subdichotomously branched cortex. In addition, the simple, laterally positioned carpogonial branch and the straight, obliquely directed trichogyne, rather unspecialized auxiliary cell branch, corn cob-like arrangement of the spermatangial heads and the predominantly decussately divided tetrasporangia are only typical for the species in this complex.

The *Gibsmithia hawaiiensis* complex together with the non-hairy *G. dotyi* and *G. larkumii* form a well-supported clade within the family Dumontiaceae. The few sequences of *G. dotyi* and *G. larkumii* currently available also indicate the existence of hidden diversity in these taxa, with two lineages in the first and three in the latter (Figs. 3–5). The inclusion of *G. womersleyi* as well as of additional sequences of

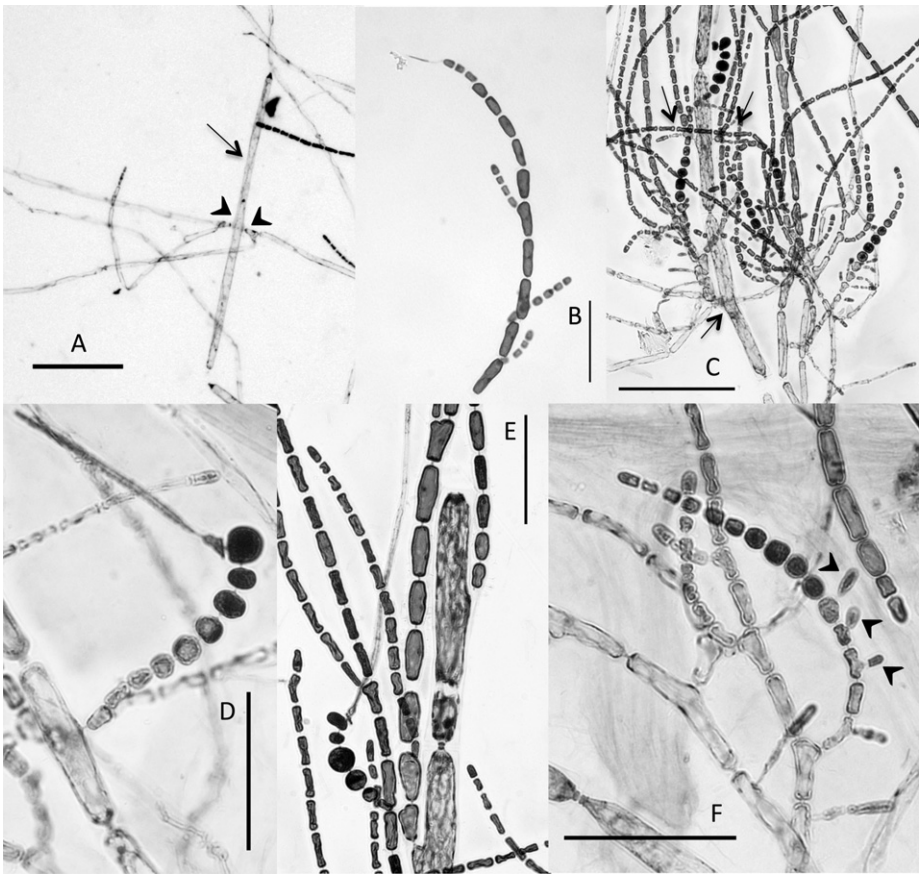

FIG. 11. *Gibsmithia indopacifica* sp. nov. (A) Narrow rhizoidal filaments located in opposite pairs on small cells (arrowheads) cut off from intercalary cells of percurrent filament (arrow). Slide SGAD1205043-2, scale bar 100 μm. (B) Hair-like structure growing on terminal cortical cell. Slide SGAD1401011-1, scale bar 50 μm. (C) Cortical filaments formed from small roundish cells (arrows) cut off from percurrent filament, and carpogonial and auxiliary cell branches in close vicinity to one another. Slide SGAD1205144-1, scale bar 100 μm. (D) Carpogonial branch with carpogonium rotated from terminal to lateral position at right angle to hypogynous cell. Slide SGAD1205144-1, scale bar 50 μm. (E) Unfertilized carpogonial branch. Slide SGAD1205144-1, scale bar 50 μm. (F) Small, narrow cells cut off unilaterally (arrowheads) from lower cells of auxiliary cell branch. Slide SGAD1205144-1, scale bar 50 μm.

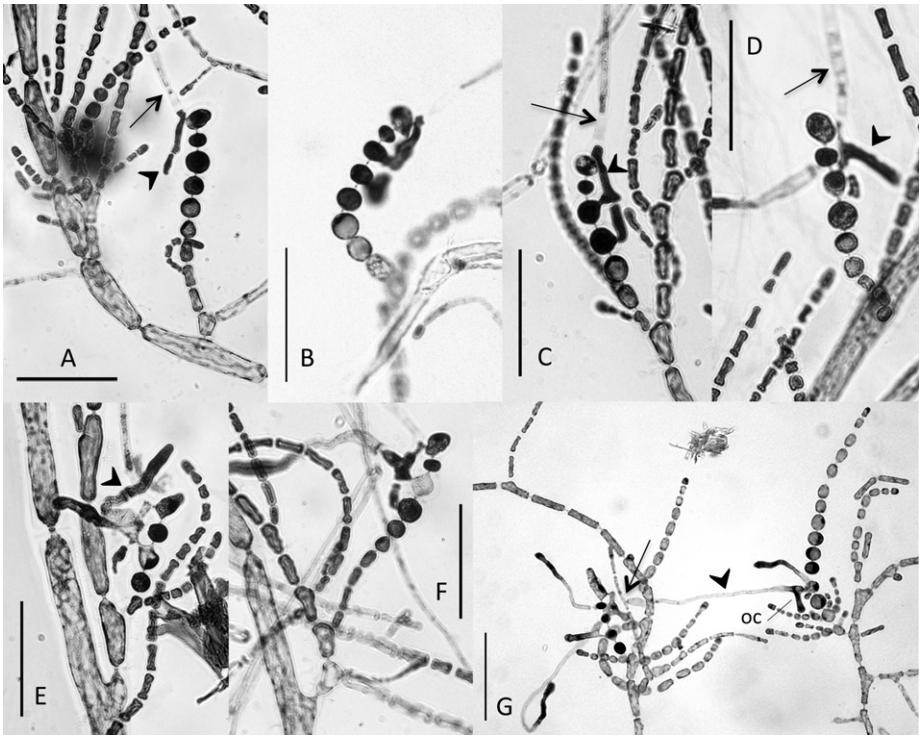

FIG. 12. *Gibsmithia indopacifica* sp. nov. Early post-fertilization stages. (A) Early post-fertilization stage showing fertilized carpogonium with trichogyne (arrow) and connecting filament primordium (arrowhead). Slide SGAD1205144-1, scale bar 50 μm. (B) Fertilized carpogonium produces a bridge that connects to the 3 cells beneath it in the carpogonial branch, divides and then connects to the fourth cell. SGAD1401011-1, scale bar 50 μm. (C) Carpogonial branch with trichogyne (arrow) and carpogonial bridge (arrowheads). Slide SGAD1205144-3, scale bar 50 μm. (D) Carpogonial branch with trichogyne (arrow), carpogonial bridge and darkly staining connecting filament initials (arrowheads). Slide SGAD1205144-1, scale bar 50 μm. (E, F) Same as in D but with septate connecting filament (arrowhead). Slide SGAD1205144-3, scale bar 50 μm. (G) Segmented connecting filaments (arrowhead) produced from the bridge (arrow) formed in the carpogonial branch after fertilization and from other cells in the carpogonial branch, fused with auxiliary cell and emitting new outgoing connecting filaments (oc). Slide SGAD1401011-1, scale bar 50 μm.

G. larkumii are necessary to further assess the diversity and monophyly of the genus.

Species of *Dudresnaya*, *Gibsmithia*, and *Kraftia* differ from other Dumontiaceae by not having highly differentiated vegetative and reproductive filaments, and by presenting a mucilaginous or lubricious habit (Lindstrom 1988). The multiaxial genus *Gibsmithia* differs from the closely related uniaxial genus *Dudresnaya* in its growth pattern (Kraft 1986), and in the cruciately divided tetrasporangia in the former versus the zonate tetrasporangia in the latter (Robins and Kraft 1985). *Gibsmithia* differs from *Kraftia* in the lack of thick-walled hyaline hairs (Shepley and Womersley 1983), and in having isomorphic generations with cruciately divided tetrasporangia (Womersley 1994), while *Kraftia* is heteromorphic with a crustose tetrasporophyte having zonate tetrasporangia (Womersley 1994).

Previous studies have suggested that *Gibsmithia* presented an isolated position within the Dumontiaceae (Shepley and Womersley 1983, Kraft 1986), not only for its unusual habit but also for forming direct fusions in addition to secondary pit-connections. This assumption is corroborated by the present study as *Gibsmithia* is phylogenetically related to other members of the Dumontiaceae but forms a distinct, well-supported clade.

Further investigations are needed to resolve the relationships between the allopatric lineages B, G, and I, as well as between the sympatric lineages E and H. Additional sampling is also necessary to proceed with the formal description of the reported putative species. This study and our earlier study (Gabriel et al. 2016) bring us closer to assess the true biodiversity of the genus *Gibsmithia*.

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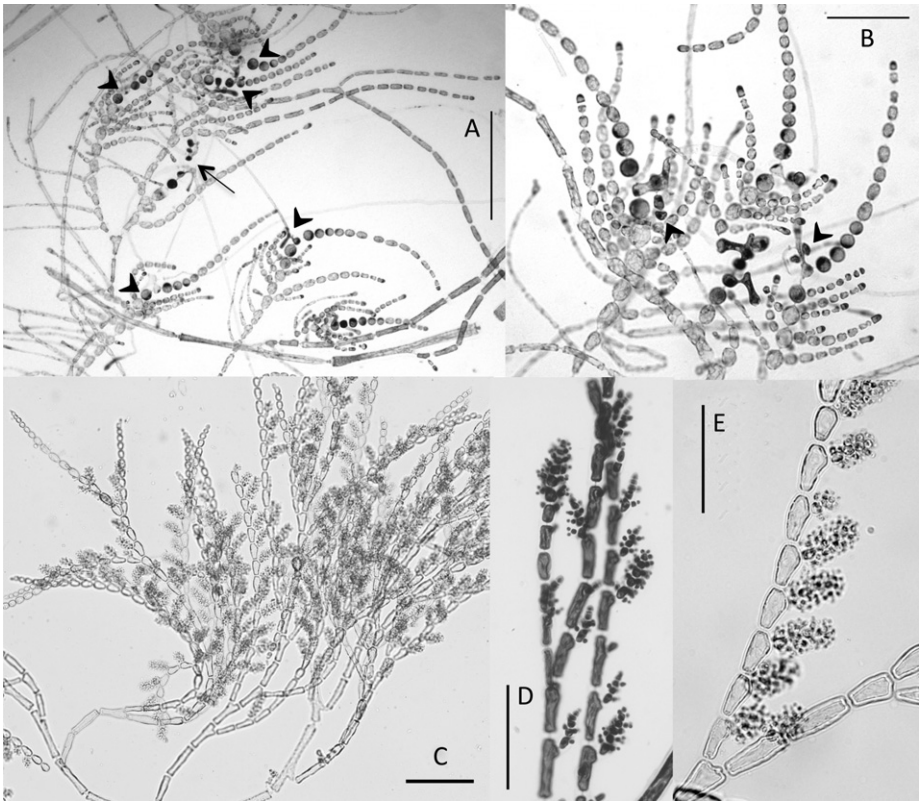

FIG. 13. *Gibsmithia indopacifica* sp. nov. Post-fertilization and male stages. (A) One fertilized carpogonial branch (arrow) and 5 auxiliary cells (arrowheads) diploidized by a presumed fertilization. Slide SGAD1401011-2, scale bar 100 µm. (B) Connecting filaments diploidizing 3 auxiliary cells. Gonimoblast initials (arrowheads) developing from the connection filament segment fused with auxiliary cell. Slide SGAD1401011-2, scale bar 50 µm. (C) Male spermatangial heads formed laterally on cortical filaments. Slide SGAD1209360-1, scale bar 100 µm. (D, E) Spermatangial head development. (D) Slide SGAD1205043-1, scale bar 50 µm. (E) Slide SGAD1209360-1, scale bar 50 µm.

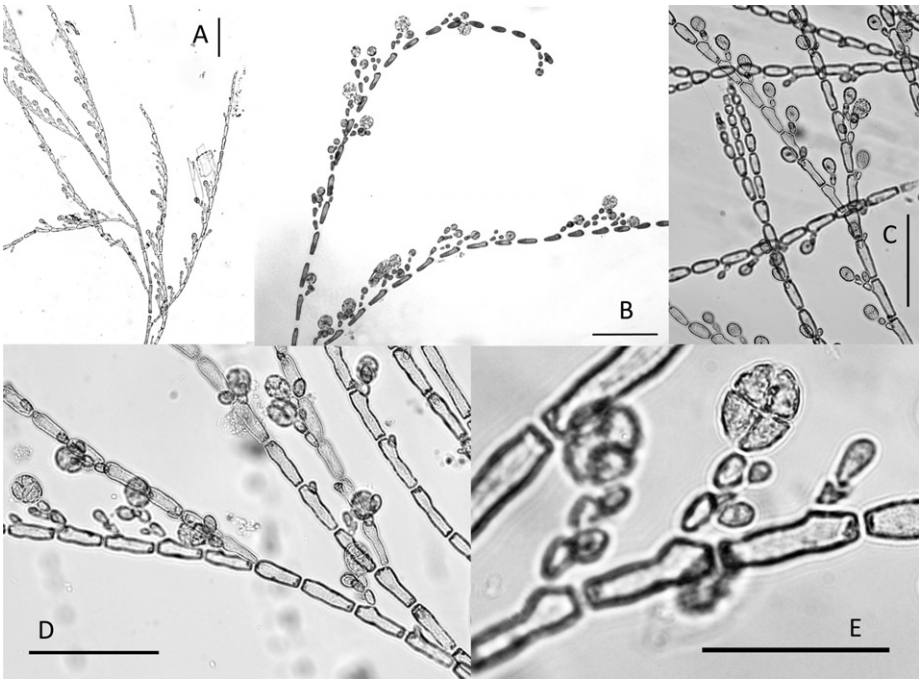

FIG. 14. *Gibsmithia indopacifica* sp. nov. Tetrasporangial initials and cruciately divided tetrasporangia. (A) Slide SGAD1205027-1, scale bar 100 µm. (B) Tetrasporangia terminal on short lateral branches, usually borne adaxially, sometimes alternate on outer cortical cells. Slide HV94-2, scale bar 100 µm. (C, D) Slide SGAD1209261-1, scale bar 100 µm. (E) Slide SGAD1209261-1, scale bar 50 µm.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web site:

Figure S1. Schematic representation of divergences as uncorrected *p*-distances (%) for each of the analyzed genes.

Figure S2. Frequency histograms of pairwise genetic distance values (uncorrected *p*-distances) across specimens belonging to the *Gibsmithia hawaiiensis* complex. (A) COI-5P. (B) *rbc*L. (C) UPA.

Figure S3. Variation of Parsimony-informative characters per site and nucleotide diversity (π) for each gene dataset, according to the taxonomic rank included.

Figure S4. Phylogenetic reconstructions of the *Gibsmithia hawaiiensis* complex as inferred from Bayesian Inference analyses of UPA sequences. Bayesian Inference posterior probabilities (PP) and Maximum Likelihood bootstrap (BS) presented as "PP/BS" near branches. * represent PP of 1.0 and BS of 100%. Newly generated sequences are presented in bold. Letters A–I represent the different mitochondrial lineages (see Fig. 3). The other ingroup taxa are members of the Dumontiaceae, the outgroup taxon is a member of the Kallymeniaceae.

Figure S5. Clustering of *Gibsmithia* specimens based on neighbor-joining analysis of *rbc*L sequences. Letters A–I represent the different mitochondrial lineages (see Fig. 3).

Figure S6. *Gibsmithia malayensis* sp. nov. Range of habits. (A–D) Herbarium-pressed. (A) Female gametophyte, SGAD1312071, Cebu, Philippines. (B) Male gametophyte, SGAD1312100, Cebu, Philippines. (C) Female gametophyte, SGAD1403107, Calamian, Philippines. (D) Sterile specimen, SGAD1404052, Camiguin, Philippines. (E) Formalin-preserved. Monoecious gametophyte, SGAD0712653, Raja Ampat, Indonesia.

Figure S7. Habit of *Gibsmithia indopacifica* sp. nov. Range of herbarium-pressed habits. (A) HV812, Luzon, Philippines. (B) DG683, Great Barrier Reef, Australia. (C) DG705, Ningaloo, Western Australia. (D) HV94, tetrasporophyte, Moorea, French Polynesia. (E) SGAD0509563,

Kepulauan Seribu, Indonesia. (F) SGAD0509657, Kepulauan Seribu, Indonesia.

Table S1. Collection data of voucher specimens and Genbank accession numbers for sequences used in this study. Newly generated sequences are presented in bold. Herbaria in which vouchers are housed are abbreviated as follows: the University of Louisiana at Lafayette Herbarium (LAF), the University of Guam Herbarium (GUAM), the Ghent University Herbarium (GENT), the Herbarium of the University of North Carolina Wilmington (WNC), the National Herbarium of the Netherlands, Naturalis (L), the State Herbarium of South Australia (AD), the University of Malaya Herbarium (KLU), and the Herbarium of the Muséum National d'Histoire Naturelle (PC). Letters A–I represent the different mitochondrial lineages (see Fig. 3).

Table S2. Diversity measures (variable sites, parsimony-informative sites, number of haplotypes and nucleotide diversity) for each gene dataset, according to the taxonomic rank included.

Table S3. COI-5P uncorrected *p*-distances within (diagonal) and between (lower left) lineages of the *Gibsmithia hawaiiensis* complex, presented as Mean (min - max) %. Letters A–I represent the different mitochondrial lineages (see Fig. 3).

Table S4. *rbc*L uncorrected *p*-distances within (diagonal) and between (lower left) lineages of the *Gibsmithia hawaiiensis* complex, presented as mean (min - max) %. Letters A–I represent the different mitochondrial lineages (see Fig. 3).

Table S5. UPA uncorrected *p*-distances within (diagonal) and between (lower left) lineages of the *Gibsmithia hawaiiensis* complex, presented as mean (min - max) %. Letters A–I represent the different mitochondrial lineages (see Fig. 3).

1 **Accepted for publication in *Journal of Biogeography***

2 # Patterns and drivers of species diversity in the Indo-Pacific red seaweed

3 # *Portieria*

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5 Running title: biogeographic history of Indo-Pacific seaweed

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ABSTRACT

Aim Biogeographical patterns and processes of Indo-Pacific biodiversity have been relatively well studied in marine shallow water invertebrates and fishes, but have been explored much less extensively in seaweeds. Using the marine red alga *Portieria* as a model, we aim to gain understanding in the evolutionary processes generating seaweed biogeographical patterns. Our results will be evaluated and compared with known patterns and processes in animals.

Location Indo-Pacific seas.

Methods Species diversity estimates were inferred using DNA-based species delimitation methods. Historical biogeographical patterns were inferred based on a six-gene time-calibrated phylogeny, distribution data of 802 specimens, and probabilistic modelling of geographic range evolution. The importance of geographic isolation for speciation was further evaluated by population genetic analyses within species.

Results We delimited 92 candidate species, most with restricted distributions, suggesting low dispersal capacity. Highest species diversity was found in the Indo-Malay Archipelago (IMA). Our phylogeny indicates that *Portieria* originated during the late Cretaceous in the area that is now the Central Indo-Pacific. The biogeographical history of *Portieria* includes repeated dispersal events to peripheral regions, followed by long-term persistence and lineage diversifications within those regions, and limited dispersal back to the IMA.

Main conclusions Our results suggest that the long geological history of the IMA played an important role in shaping *Portieria* diversity. High species richness in the IMA resulted from a combination of speciation at small spatial scales, possibly as a result of increased regional habitat diversity from the Eocene onwards, and species accumulation via dispersal and/or island integration through tectonic movement. Our results are consistent with the biodiversity feedback model, in which biodiversity hotspots act as both ‘centres of origin’ and ‘centres of accumulation’, and corroborate previous findings for invertebrates and fish that there is no single unifying model explaining the biological diversity within the IMA.

Keywords biodiversity hotspot, Coral Triangle, cryptic species, historical biogeography, algae, marine biogeography, Rhodophyta, speciation, Indian Ocean

INTRODUCTION

A wide range of marine organisms (including coastal fishes, several invertebrate groups, and marine angiosperms) reach their highest species richness in the tropical region bounded by the Philippines, Indonesia and Papua New Guinea, known as the Coral Triangle or Indo-Malay Archipelago (IMA). Diversity declines rapidly for most groups when moving away longitudinally as well as latitudinally from the IMA (Connolly *et al.*, 2003; Hoeksema, 2007). Marine macroalgae (seaweeds) are among the dominant groups of benthic organisms in nearshore marine environments but are a contrarian group because their biogeographic patterns of community assembly and diversity differ from those of most marine taxa (Schils *et al.*, 2013; Etti & Schils, 2016). A number of seaweed groups, including brown seaweeds and siphonous green algae, however, display a similar pattern of peak diversity in the IMA (Kerswell, 2006; Vieira *et al.*, 2017).

The high species richness in the IMA has intrigued evolutionary biologists for decades, and several competing but non-exclusive hypotheses have been proposed to explain the origins of this marine biodiversity hotspot, including the centre of origin, the centre of accumulation, and the region of overlap hypotheses. The relative importance of these models, however, remains a matter of controversy (Barber, 2009; Bellwood & Meyer, 2009; Jablonski *et al.*, 2013).

The centre of origin hypothesis suggests that the high diversity is due to elevated speciation rates within the IMA as a consequence of geological complexity, habitat heterogeneity and intense competition within the region. In this model, dispersal of species to peripheral regions has resulted in a pattern of declining diversity with distance away from the centre (Briggs, 2000; Mora *et al.*, 2003). There is evidence that tectonic events such as the collision of the Australia-New Guinea plate with SE Eurasia resulted in increased diversification in the Oligo-Miocene (Williams & Duda, 2008).

The centre of accumulation hypothesis suggests that the high number of species in the Coral Triangle is a result of speciation in peripheral locations, with subsequent dispersal and accumulation of species in the IMA (Jokiel & Martinelli, 1992). In this model, the biodiversity hotspot is explained by lower extinction rates in the IMA, mediated by its extensive and heterogeneous tropical shallow-water environments with large reef areas (Barber & Bellwood, 2005; Bellwood & Meyer, 2009). Accumulation of species may also have resulted from integration of distinct biotas by tectonic movement over the past 50 million years (Rosen & Smith, 1988; Hall, 2002; Renema *et al.*, 2008).

The region of overlap hypothesis suggests that the high species diversity results from overlap of species ranges due to vicariance events and subsequent range expansion across the IMA (Barber *et al.*, 2000; Bellwood & Wainwright, 2002).

Phylogenetic and population genetic data of marine invertebrates and fish have provided evidence in support of all three hypotheses: centre of origin (e.g., Carpenter & Springer, 2005; Barber *et al.*, 2006; Tornabene *et al.*, 2015; Ukuwela *et al.*, 2016), centre of accumulation (e.g., Drew & Barber,

2009; Eble *et al.*, 2011; Hodge *et al.*, 2012), and region of overlap (e.g., Gaither *et al.*, 2011; Hubert *et al.*, 2012). This indicates that several processes likely contributed to the IMA biodiversity hotspot for different taxa (Bowen *et al.*, 2013; Hodge & Bellwood, 2016; Ukuwela *et al.*, 2016).

The fossil record indicates that the IMA has not always been a centre of marine biodiversity. During the past 50 million years, marine biodiversity hotspots have shifted from the West Tethys in the area that is now the Mediterranean Sea and the Red Sea, to the northern Indian Ocean, and finally the IMA today, mirroring the regions that had large areas of shallow water and suitable climatic conditions at various stages in earth history (Renema *et al.*, 2008). Concurrently, historical biogeographical analyses of coral reef fishes suggest that the importance of the Central Indo-Pacific has changed from an area of species accumulation in the Paleo/Eocene, to a centre of origination since the Miocene (Cowman & Bellwood, 2013b; Cowman, 2014).

Historical biogeographical studies investigating patterns of species origin and dispersal in the tropical Indo-Pacific have largely focused on marine animals, and relatively few studies (mainly on fish) have analysed species-rich groups across large geographical scales (Barber & Bellwood, 2005; Gaither *et al.*, 2011; Ukuwela *et al.*, 2016). Despite being a diverse and major component of tropical coastal ecosystems, seaweeds have not received much attention in historical biogeographical studies in the Indo-Pacific, and in addition biogeographical patterns have been largely obscured by rampant cryptic diversity (Vieira *et al.*, 2017). Compared to marine fish and invertebrates with planktonic larvae, most seaweeds are poor dispersers because their spores and zygotes are typically short-lived and negatively buoyant (Kinlan & Gaines, 2003). As a result, many seaweed species have restricted geographic ranges and molecular data indicate that several allegedly widely distributed species in fact represent cryptic species with narrow distributions (e.g., Zuccarello & West, 2003; Saunders, 2005; Gabriel *et al.*, 2017).

We chose the red seaweed *Portieria* (family Rhizophyllidaceae, order Gigartinales) to study patterns of species origination and dispersal in the tropical Indo-Pacific because (1) it is a common alga in nearshore marine environments of the tropical Indo-Pacific region (Guiry & Guiry, 2018), (2) the genus is species-rich (Payo *et al.*, 2013), (3) its vegetative and reproductive development have been well studied (Payo *et al.*, 2011), and (4) it is easily recognizable in the field by its typical branching pattern, facilitating identification and collection. *Portieria* is commonly found on coral reefs and rocky shores where it grows in the intertidal, and subtidally to 40 m deep. Because *Portieria* species grow attached and lack obvious propagules, dispersal capacity is expected to be limited. About five species of *Portieria* have traditionally been recognized based on morphological criteria (Wiseman, 1973; Masuda *et al.*, 1995; De Clerck *et al.*, 2005; Anderson *et al.*, 2016). One of these, *P. hornemannii*, is thought to have a broad distribution from the northern Red Sea to French Polynesia (Guiry & Guiry, 2018), which contradicts with the idea of poor dispersal capacity. A biodiversity study in the Philippine archipelago based on DNA sequence data, however, showed that 21 cryptic

114 species, all with very narrow distribution ranges, were contained within the *P. hornemannii* morpho-
115 species complex (Payo *et al.*, 2013). This discovery indicates that the global species diversity in the
116 genus is probably much higher and makes the genus a good candidate to study global patterns of
117 diversity and the processes underlying them.

118 Because an accurate knowledge of species boundaries and distributions is important for
119 evolutionary inference, the first aim of our study was to assess species diversity and geographical
120 distributions of *Portieria* in the Indo-Pacific based on DNA sequence data. Building upon these
121 results, our main goal was to investigate patterns of species origin and dispersal by modelling
122 geographic range evolution using a time-calibrated phylogenetic framework. The importance of
123 geographic modes of speciation in the diversification of *Portieria* was further evaluated by analysis of
124 population genetic structure within well sampled species. Our results were evaluated in light of current
125 hypotheses explaining the origins of the IMA marine biodiversity hotspot and were compared to
126 studies on fish and invertebrates to explore (dis)similarities with processes found in marine animals.

127

128 **MATERIALS AND METHODS**

129 **Sampling and laboratory protocols**

130 We sampled 999 specimens of *Portieria* from 260 localities, encompassing most of the
131 geographical range of the genus (Fig. S1 in Appendix S1 in Supporting Information). The list of
132 specimens with collection data and voucher information is provided in Table S1 in Appendix S1.

133 DNA extraction, PCR amplification and sequencing protocols are detailed in Table S2 in Appendix
134 S2. For species delimitation, we targeted the mitochondrial *cox2-3* spacer (363 bp). This marker was
135 successfully sequenced for 802 specimens. For constructing a species phylogeny, the *cox2-3* spacer
136 was complemented with five additional markers: the mitochondrial encoded *cox1* gene (642 bp), the
137 plastid encoded *psbA* gene (939 bp), *rbcL* gene (1027 bp) and *rbcL-rbcS* spacer (537 bp), and the
138 nuclear encoded elongation factor 2 (*EF2*) gene (in two parts: 474 bp and 609 bp). Sequences have
139 been submitted to the EMBL/GenBank databases under accession numbers ***_***.

140

141 **DNA-based species delimitation and geographical distributions**

142 We applied three approaches to species delimitation based on the *cox2-3* spacer dataset: statistical
143 parsimony (Templeton *et al.*, 1992), single and multiple threshold Generalized Mixed Yule Coalescent
144 approach (GMYC) (Pons *et al.*, 2006; Monaghan *et al.*, 2009), and a Poisson Tree Processes (PTP)
145 model approach (Zhang *et al.*, 2013). Details of the species delimitation analyses are provided in
146 Appendix S3.

147 Species distributions, based on locations of the 802 sequenced specimens, were plotted with the
148 'maps' package in R (cran.r-project.org/web/packages/maps/). Geographic patterns of species richness
149 were based on the numbers of species recorded in 12 marine biogeographic provinces, modified by
150 Spalding *et al.* (2007). Latitudinal and longitudinal range sizes of each species were calculated as
151 described in Baselga *et al.* (2012).

152

153 **Multi-locus time-calibrated species phylogeny**

154 A species phylogeny was based on an alignment of the delimited *Portieria* species (each represented
155 by a single specimen) and six markers: *cox2-3* spacer, *cox1*, *psbA*, *rbcL*, *rbcL-rbcS* spacer, and *EF2*,
156 with the different markers coming from the same specimen. DNA sequences were aligned for each
157 marker separately using MUSCLE (Edgar, 2004) with amino acid translations taken into account for
158 protein coding regions. The six alignments were then concatenated into a single alignment of 3,782
159 positions, which was 71% filled at the species × locus level. Information on sequence alignments is
160 given in Table S3 in Appendix S2.

161 PARTITIONFINDER (Lanfear *et al.*, 2012) was used to identify a suitable partitioning scheme and
162 accompanying substitution models according to the Bayesian information criterion (BIC) based on a
163 set of eight *a priori* defined partitioning schemes. Three partitioning schemes (3, 5 and 8 data
164 partitions) were selected for the phylogenetic analyses (Table S5 in Appendix S4).

165 The age of the root of the *Portieria* clade was estimated based on the red algal time-calibrated
166 phylogeny of (Yang *et al.*, 2016). We assembled a seven-gene dataset of Gigartinales and
167 Peyssonneliales and complemented this dataset with genera of Rhizophyllidaceae, including nine
168 representatives of the main *Portieria* clades. Genes were aligned as described above, and a time-
169 calibrated tree was estimated with BEAST v1.8.2 (Drummond *et al.*, 2012). The root of the tree (split
170 between Gigartinales and Peyssonneliales) was constrained with a normal prior distribution (mean =
171 308 Ma, SD = 23) based on Yang *et al.* (2016). Using this calibration, the crown age of *Portieria* was
172 estimated at 99.2 Ma (Fig. S4 in Appendix S4), which was used to obtain a time-frame of
173 diversification for the genus *Portieria* in the BEAST analysis described below.

174 A time-calibrated Bayesian phylogeny of *Portieria* was constructed with BEAST based on the
175 concatenated six-marker alignment. The three partitioning schemes were used with the unlinked
176 GTR+I+G model for each partition. Data were analysed using a Birth-Death tree prior (Gernhard,
177 2008), an uncorrelated lognormal (UCLN) relaxed clock model of rate variation among branches
178 (Drummond *et al.*, 2006) with the mean of the branch rates (ucld.mean) constrained with a diffuse
179 gamma distribution prior (shape 0.001, scale 1000). All other priors were left as default. The root of
180 the tree, being the crown node of *Portieria*, was constrained with a normal prior distribution (mean =
181 99 Ma, SD = 10). Four independent MCMC analyses of 20 million generations were performed,

sampling every 2,000 generations, to obtain posterior distributions of parameters excluding a burnin of 10%. Convergence of each analysis was determined in TRACER v.1.6 (Rambaut *et al.*, 2014), examining the effective sampling size for all parameters. For the analysis using three data partitions, the effective sampling size (ESS) was > 200 for all parameters (except for the GTR substitution parameters of codon positions 1+2 with ESS 100-200), while for the analyses with five and eight data partitions, convergence was poor (ESS < 100) for several of the GTR substitution parameters. MCMC analyses were combined in LOGCOMBINER v1.8.2, and maximum clade credibility trees were generated with TREEANNOTATOR. FIGTREE v1.4.2 (Rambaut, 2014) was used to visualize the chronogram. Analyses using the three different partitioning schemes led to similar tree topologies and resolution as well as similar time estimates.

Inference of biogeographic history

The time-calibrated species phylogeny and the geographic ranges of the species were combined to analyse the historical biogeography of *Portieria*. Ancestral ranges were estimated using BIOGEOBEARS (Matzke, 2013), an R package implementing several ancestral range estimation models in a likelihood framework, including the Dispersal-Extinction Cladogenesis Model (DEC) (Ree & Smith, 2008), a likelihood version of the parsimony-based Dispersal-Vicariance Analysis (Ronquist, 1997) (DIVALIKE), and a likelihood version of the range evolution model implemented in the BayArea program and the Bayesian Binary Model (BBM) of RASP (Yu *et al.*, 2015). It also includes the possibility to incorporate the process of founder-event speciation (+J) to the above-mentioned models.

Two geographical subdivisions were considered. In the realm-level analysis, three broadly defined realms modified from Spalding *et al.* (2007)) are considered: a-c in Fig. 1. In the province-level analysis, twelve provinces (modified from Spalding *et al.* (2007)) are considered: A-L in Fig. 1. Geographical distributions were based on location data of the 802 sequenced specimens.

For both geographical subdivisions, the six different models implemented in BIOGEOBEARS were compared for statistical fit using the Akaike Information Criterion (AIC) (Table S6 in Appendix S5). The maximum number of areas for a single species to occupy was set at two and three for the realm- and province-level analysis, respectively. The best-fit model was then used to refine the analysis with constrained areas and dispersal multipliers in which dispersal probability decreased with geographical distance (Table S7 in Appendix S5). The resulting ancestral range probability for each node was plotted on the BEAST tree. In the province-level analysis, biogeographic event counts, and probabilities of events at each node were determined using Biogeographic Stochastic Mapping in BIOGEOBEARS under the best-fit model, DEC+J.

Shifts in diversification rate through time and among lineages were tested using BAMM (Rabosky, 2014), using the BEAST tree as input, expected number of shifts = 1, with 100 million generations of Markov Chain Monte Carlo (MCMC) sampling per run and sampling evolutionary parameters every 100,000 generations. A lineages-through-time (LTT) plot, including a 95% confidence interval based on a set of 1,000 post-burnin trees was generated using PHYTOOLS (Revell, 2012).

Population genetic analysis

Within species, we assessed if populations were geographically structured using haplotype network analyses and single-level Analysis of Molecular Variance (AMOVA). For these analyses, we selected 19 species for which 10 or more specimens were available from at least two geographically distinct locations (≥ 20 km apart), and with a minimum of two specimens per population. For four additional species only haplotype networks were constructed. Haplotype networks of *cox2-3* spacer sequences were built using the TCS method (Clement *et al.*, 2000) with POPART v.1.7 (Leigh & Bryant, 2015). AMOVA and fixation index Φ_{st} calculations, using 1,000 permutations were performed in ARLEQUIN v3.5.2 (Excoffier & Lischer, 2010). Because of limited sampling in many populations, we did not calculate pairwise Φ_{st} values between populations, and AMOVA results should be considered as indicative.

RESULTS

Species diversity and geographic ranges

Results of the different DNA-based species delimitation analyses are summarized in Table S4 and Fig. S2 in Appendix S3. The different methods yielded species diversity estimates ranging from 81 (statistical parsimony) to 139 species (multiple threshold GMYC). Because the GMYC and PTP methods are known to overestimate species numbers in some cases (for example when taxon sampling is uneven or incomplete), we relied on a conservative consensus approach towards reconciling the results of the different species delimitation methods to maximize the reliability of species boundaries, as has been suggested in other studies (Carstens *et al.*, 2013; Miralles & Vences, 2013; Zhang *et al.*, 2013). More specifically, we recognized species clades that received high support in the *cox2-3* spacer BEAST tree (posterior probabilities > 0.95), and that were compatible with at least three of the four species delimitation methods (statistical parsimony, GMYC single, GMYC multiple and PTP). This resulted in the delimitation of 92 candidate species of *Portieria*. Species delimitations were generally congruent with the results of Payo *et al.* (2013), which only included Philippine data. One exception is the subclade including V1A-V1B-V1C, which was split into three species based on analysis of multi-

locus data under a multispecies coalescent model in Payo *et al.* (2013), but is here regarded as a single unit, underscoring our conservative approach towards species delimitation.

Although a number of species names are available in the genus *Portieria*, we do not apply these names at this stage because in most cases they could not be reliably applied to any of the 92 candidate species. One exception is *P. tripinnata* from South Africa, which grows in the mid-intertidal (De Clerck *et al.*, 2005; Anderson *et al.*, 2016), and most likely corresponds to sp.32.

The geographical distributions of the 92 *Portieria* species are summarized in Fig. 1 and Fig. S3 in Appendix S3. In the realm-level analyses (3 realms), each species was restricted to a single realm, with most species (72) occurring in realm b (Central Indo-Pacific, Temperate Northern Pacific and Temperate Australasia). In the province-level analyses (12 provinces), most species (81) were restricted to a single province, 10 species occurred in two provinces, and only one species spanned three provinces (sp.34 occurring in provinces B, C and D). Most species thus have narrow geographical ranges, being restricted to single island groups or short coastal stretches. Latitudinal and longitudinal range sizes of the different species are illustrated in Fig. 2. Most species (77 of the 92) had a latitudinal and/or longitudinal range smaller than 500 km, and only 7 species had a latitudinal and/or longitudinal range larger than 2,000 km. As an exception, sp.34, which is found from South Africa to Oman, as well as in Madagascar and Sri Lanka, had latitudinal and longitudinal ranges exceeding 5,000 km.

Highest species diversity was observed in the Western Coral Triangle (F), including 31 species (Fig. 1), followed by the Western Indian Ocean (B), the Southwestern Pacific (J), the Northwestern Pacific (I), and the Eastern Coral Triangle (G) (each containing 9 to 14 species). Observed species diversity in the other provinces was much lower (2-4 species). We found a marginally significant correlation between the number of specimens sampled and number of species found per province (r_s = 0.632, P = 0.027), thus the effect of sampling effort on species richness cannot be ruled out entirely.

Within provinces, most sister species showed non-overlapping ranges (Fig. S3 in Appendix S3), concordant with the results of Payo *et al.* (2013).

Biogeographic history

The time-calibrated phylogeny (Fig. 3) recovered several well supported clades (PP>0.95) originating from the Late Cretaceous onwards (for convenience, ten main clades, I to X, are indicated). The rate of diversification within the genus was relatively constant across time, with neither the LTT plot nor the BAMM analysis showing evidence for rate shifts (Fig. S5 in Appendix S4).

Comparisons between historical biogeographical models showed that the incorporation of founder event speciation (+J) in the models yielded a significantly better fit (Table S6 in Appendix S5). In both

283 the realm- and province-level analyses, the DEC+J model was favoured based on the AIC, although
284 the likelihood differences with the DIVALIKE+J and the BAYAREALIKE+J models were small. The
285 inferred province-level biogeographic history is shown in Fig. 3; the inferred realm-level
286 biogeographic history is shown in Fig. S7 in Appendix S5.

287 Most main clades were confined to a single or a few adjacent realms or provinces. In the realm-
288 level analysis, the ancestral range was inferred as a or ab under the DEC+J model (Fig. S7 Appendix
289 S5), and realm b under the DIVALIKE+J and BAYAREALIKE+J models (data not shown). In the
290 province-level analysis, the ancestral range of *Portieria* was inferred as F, FI, FJ or FIJ, corresponding
291 to the area that is now the Central Indo-Pacific, Northern Pacific and Australasia (Fig. 3).

292 Biogeographic stochastic mapping (province-level analysis) indicated within-province speciation
293 ("narrow sympatry") as the most important event in the history of the group, and an intermediate
294 number of nodes were inferred to represent founder event speciation (Fig. 4, Fig. S8 in Appendix S5).
295 Vicariance, subset sympatry (sister species being sympatric across part of their range), and anagenetic
296 dispersal (range expansion of a species) were of lesser importance. However, anagenetic dispersal,
297 followed by subset sympatry was inferred along several branches in clade IX, including species from
298 Temperate Southern Africa, Western Indian Ocean, and Somali/Arabia (provinces A, B and C) (Fig.
299 S8 in Appendix S5).

300 Our analyses show highest diversification within the Western Coral Triangle (province F), and
301 repeated species export to the Northwestern Pacific (I), Western Indian Ocean (B), and Southwestern
302 Pacific (J) (Fig. 5). Dispersal to the Western Indian Ocean, and the North- and Southwestern Pacific
303 was followed by diversification within those provinces, and dispersal from the North- and
304 Southwestern Pacific back to the Coral Triangle. Conversely, species from the Western Indian Ocean
305 did not disperse back to the Central Indo-Pacific. The origin of *Portieria* species on remote islands,
306 including Micronesia (sp.67 and sp.68), Guam (sp.54) and Hawaii (sp.69), could not be inferred with
307 certainty, either because phylogenetic relationships were uncertain or because inferred ancestral
308 geographic ranges were ambiguous. *Portieria* sp.42 from Hawaii may have a Southwestern Pacific
309 origin.

310

311 **Population genetic structure within species**

312 Within 13 of the 19 species analysed, analysis of genetic variation of the *cox2-3* spacer indicated
313 significant population genetic structuring. Significant geographic structuring of populations was
314 observed from small spatial scales (< 500 km, e.g. species B21, S39 and V1ABC in the Philippines,
315 and sp. 60 in Japan) to larger spatial scales (> 1,500 km, e.g. sp. 28, sp. 34 and sp. 36 in the Western
316 Indian Ocean, sp. 46 in Indonesia, and sp. 78 in Australia) (Fig. S9 in Appendix S6). Non-significant
317 population genetic structuring was mainly found in species with small to medium sized ranges (25-700

318 km). However, no significant correlation was found between fixation index (Φ_{st}) and geographical
319 scale (calculated as maximum distance between the populations) (rs = 0.179, P = 0.464) (Table S8 in
320 Appendix S6).

321

322 **DISCUSSION**

323

324 **High species diversity and narrow ranges**

325 A first striking outcome of this study is the high number of unrecognized species in the genus
326 *Portieria*. We delimited 92 species based on *cox2-3* spacer sequence data from 802 specimens from
327 260 localities, encompassing most of the geographical range of the genus. In stark contrast, only five
328 species of *Portieria* are currently described, one of which, *P. hornemannii*, is considered to be widely
329 distributed in the Indo-Pacific (De Clerck *et al.*, 2005). A first indication that species diversity in the
330 genus is far greater than assumed based on formally described taxa was provided by Payo *et al*. (2013)
331 who recognized, based on multi-locus DNA sequence data, 21 cryptic species of *P. hornemannii*
332 within the Philippines. Although cryptic diversity is no exception in the marine environment, and in
333 seaweeds in particular (e.g., Zuccarello & West, 2003; Saunders, 2005; Pardo *et al.*, 2014; Vieira *et*
334 *al.*, 2017), the degree of cryptic diversity found in *Portieria* is remarkable.

335 There are two reasons to interpret our species-diversity estimate with some caution. Firstly, despite
336 our broad geographic sampling, some regions where *Portieria* has been recorded were not sampled in
337 our study, including the Red Sea, Bay of Bengal, and several remote Islands in the Pacific, including
338 French and Central Polynesia, the Solomon Islands, Fiji, and the Northern Mariana Islands (Guiry &
339 Guiry, 2018). If the observed narrow distributions of *Portieria* species can be extrapolated, sampling
340 these regions is likely to further increase species numbers. Secondly, our analyses were based on
341 single-locus data, which cannot take into account processes such as incomplete lineage sorting that can
342 possibly confound species delimitation (Leliaert *et al.*, 2014). However, the fact that our species
343 delimitations were highly concordant with the results of Payo *et al*. (2013), which were based on three
344 unlinked loci from the nucleus, mitochondrion and chloroplast, increases confidence in our estimates
345 of species boundaries.

346 Our study shows that with a few exceptions *Portieria* species have narrow, often very narrow,
347 geographic ranges (Fig. 2) refuting the reported wide distribution of *P. hornemannii* across the entire
348 Indo-Pacific. Instead, all species are confined to a single Ocean basin, and most species are restricted
349 to short stretches of coastline or single archipelagos. Our results corroborate earlier findings of fine-
350 scale intra-archipelagic endemism within the Philippines, indicating limited dispersal potential of
351 *Portieria* species (Payo *et al.*, 2013). In contrast, many tropical shallow-reef animals have much wider
352 species ranges within the Indo-Pacific or even span different ocean basins with high population

genetic connectivity (e.g., Paulay & Meyer, 2002; Crandall *et al.*, 2008; Pinzón *et al.*, 2013). This pattern, however, is by no means universal as many species of marine invertebrates and fish are range-restricted in remote peripheral archipelagos, or even in regions of the Central Indo-Pacific (Meyer *et al.*, 2005; Malay & Paulay, 2009; Tornabene *et al.*, 2015). The scale of endemism found in some *Portieria* species in the IMA, however, has never been recorded in animal taxa.

Coral Triangle biodiversity hotspot

We detected a clear pattern of highest species diversity in the Coral Triangle (40 recorded species) and lower diversity in peripheral regions, although species diversity is also considerable in the Western Indian Ocean, Southwestern Pacific, and Northwestern Pacific (9-14 species in each region) (Fig. 1). Similar patterns of maximum species diversity in the Coral Triangle have been observed in a broad range of tropical marine animal groups (Hoeksema, 2007; Tittensor *et al.*, 2010) and some macro-algae (Kerswell, 2006; Silberfeld *et al.*, 2013; Vieira *et al.*, 2017). Although in some groups of reef fishes this diversity peak is correlated with a high number of endemic species (Tornabene *et al.*, 2015), for many other animal groups, including corals and fishes, high diversity does not necessarily correlate with small species ranges or high endemicity. Instead, the high species richness in the IMA is often a result of strongly skewed range distributions that overlap in the IMA, thus generating a peak in species richness (Hughes *et al.*, 2002).

Diversity in the Western Coral Triangle (31 species) was found to be higher than in the Eastern Coral Triangle (9 species). A similar pattern has been observed for shore fish, and has been attributed to higher habitat availability and heterogeneity in the Western Coral Triangle (Carpenter & Springer, 2005). The pattern in *Portieria*, however, may reflect sampling effort, and additional collections from Papua New Guinea and the Solomon Islands may reveal a gradient rather than a steep decline in diversity.

Geographic mode of speciation

The strong geographic signal observed in our species phylogeny and the significant population genetic structure found within several *Portieria* species indicate that geographic modes of speciation have played an important role in the diversification of the genus. The prevalence of small species ranges and non-overlapping distributions of sister species in *Portieria*, indicate that genetic divergence and speciation can occur over very small spatial scales (< 100 km). Although geographic speciation on small spatial scales has been inferred in some tropical marine fishes and gastropods (Meyer *et al.*, 2005; Worheide *et al.*, 2008; Tornabene *et al.*, 2015), allopatric speciation in most marine animals occurs in response to barriers operating at much larger geographical scales, spanning large ocean

387 regions or even different ocean basins (Frey, 2010; Claremont *et al.*, 2011; Ahti *et al.*, 2016; Waldrop
388 *et al.*, 2016).

389 In the western Indian Ocean a few *Portieria* species have a remarkably wide distribution (e.g. spp.
390 34 and 36). Within these widely ranging species, our population genetic analyses indicate geographic
391 structure as well (Fig. S9 in Appendix S6). Thus, depending on where precisely the species limits are
392 placed, geographic partitioning is situated within a species or between species, indicating that low
393 dispersal is present at all levels. Other western Indian Ocean species are restricted to peripheral
394 regions in the SW or NW Indian Ocean. Several anagenetic dispersal events followed by subset
395 sympatry, inferred in our historical biogeographical analysis, suggest repeated segregation of these
396 peripheral species from large-ranged parent species, indicative of a peri- or parapatric speciation.
397 Although founder speciation cannot be ruled out entirely, a possible scenario involves repeated
398 speciation along a temperature gradient, in which species expand their ranges north- or southwards,
399 followed by local adaptation of peripheral populations to lower temperatures. Similar speciation
400 modes have been proposed for reef fishes (Hodge *et al.*, 2012; Tornabene *et al.*, 2015), and hermit
401 crabs (Malay & Paulay, 2009).

402 Cases of sympatric sister species are restricted to the Philippines (clade B33, B34 and B35) with
403 species co-occurring on the same island or even in the same locality. Although for other Philippine
404 *Portieria* species, non-overlapping ranges, and significant population genetic structuring hints toward
405 allopatric speciation within the archipelago (Payo *et al.*, 2013), it is difficult to untangle sympatric
406 speciation from allopatric divergence on small spatial scales, possibly followed by subsequent
407 dispersal and colonization events or secondary sympatry (Andersen *et al.*, 2015). Sympatric or
408 parapatric speciation along ecological boundaries in the marine environment has been inferred from a
409 growing body of phylogenetic, biogeographic and ecological data (Bowen *et al.*, 2013; Hodge *et al.*,
410 2013; Tornabene *et al.*, 2015). Sympatric speciation should not be ruled out for *Portieria*, and will
411 need to be further studied using population genetic and ecological data of co-occurring species clades
412 to evaluate the role of ecological partitioning in speciation on small geographic scales.

413

414 **Diversification and historical biogeography of an ancient genus**

415 The phylogenetic analyses indicate a late Cretaceous origin of *Portieria*. Our time estimates, however,
416 have to be interpreted with care since they are derived from the scarce fossil record of red algae and
417 thus entail some uncertainty (Yang *et al.*, 2016). A Cretaceous origin would imply that the early
418 diversification of the genus pre-dated the physical separation of the Indo-Pacific from other
419 biogeographical regions through the final closure of the Tethys Sea (18-19 Ma). Similar distribution
420 patterns have been observed for several ancient groups of Indo-Pacific animals, including gastropods

with Indo-Pacific clades that diversified 20 to 70 Ma (Williams & Reid, 2004; Williams, 2007; Williams & Duda, 2008).

Our historical biogeographical analyses indicates the area that is now the Central Indo-Pacific to be the likely geographical origin of *Portieria*, which may thus correspond to the tropical shallow reef regions of northern Australia and/or eastern Asia in the Cretaceous. The overwhelmingly tropical genus managed to invade warm temperate regions several times independently, including South Africa, Japan, Korea, and Australia, over a broad time interval in the late Paleogene and Neogene, which are periods of globally decreasing temperatures (Zachos et al., 2001). Similarly, phylogenetic analyses have indicated that the green seaweed *Halimeda* and the brown seaweed *Lobophora* managed to get across the tropical temperature barrier over similar time periods (Verbruggen *et al.*, 2009; Vieira *et al.*, 2017).

Despite the antiquity of *Portieria*, our analyses do not indicate that relict taxa (which would be recognizable as early branching species in the phylogeny) occur in the NW Indian Ocean, which could indicate past high diversity in the western Tethys, as has been demonstrated based on the fossil record and molecular phylogenetic data of various marine groups such as mangroves, benthic foraminifera, gastropods, fishes and corals (Renema *et al.*, 2008; Cowman, 2014; Leprieur *et al.*, 2016; Obura, 2016). Instead, the current diversity of the NW Indian Ocean likely originated more recently, following dispersal from the East African coast and Central Indo-Pacific.

Diversification of *Portieria* occurred relatively constantly over time, similar to what has been inferred for the brown alga *Lobophora*, a pantropical genus with comparable age to *Portieria* (Vieira *et al.*, 2017), although it should be noted that these analyses are prone to sampling bias (Pennell *et al.*, 2012). In contrast, phylogenetic data and spatial diversification models of several marine tropical animal groups have provided evidence for increased diversification in the late Cretaceous (Leprieur *et al.*, 2016) or in the Oligo-Miocene as a consequence of tectonic changes in the Central Indo-Pacific resulting in increased geographical complexity of the region, formation of island barriers, and increased availability of shallow water habitats (Wilson & Rosen, 1998; Williams & Duda, 2008). Other studies have shown accelerated speciation rates in the late Pliocene and Pleistocene, associated with periods of glacially lowered sea level when seas became land-locked, resulting in prolonged geographical isolation and the creation of empty niches (Carpenter & Springer, 2005; Crandall *et al.*, 2008; Tornabene *et al.*, 2015; Ukuwela *et al.*, 2016). We do not find evidence for similar shifts in diversification rates in *Portieria*. A possible explanation is that because of the low dispersal potential of *Portieria*, geographical isolation is accomplished as easily in periods with high sea levels than it is in periods with low sea levels for other organisms.

Our *Portieria* phylogeny provides evidence relevant to the mechanisms that produced current biodiversity patterns, including the IMA diversity hotspot. The historical biogeographical analyses

indicate that current geographical patterns of *Portieria* species resulted from long-term persistence and diversification of clades in confined regions, combined with infrequent but successful long distance dispersal events across the Indo-Pacific.

The estimated ages of the IMA clades range between 15 and 45 Ma, a time-frame that is consistent with the long-term geological formation of the IMA, and the emergence of the IMA biodiversity hotspot (Hall, 2002; Renema *et al.*, 2008). Our historical biogeographical reconstruction indicates that the high diversity of *Portieria* species in the IMA mainly resulted from extensive diversification within the region, and to a lesser extent from accumulation of species. The high availability of shallow-water habitats in the IMA likely allowed for long-term persistence of species, and, in addition, the complex geological history of the region provided opportunities for diversification, although, as mentioned above, these did not result in significant shifts in diversification rates. Our results are thus consistent with both the centre of origin and centre of accumulation models, acting over long temporal scales. Similarly, long evolutionary histories within the Central Indo-Pacific have been inferred for fishes and invertebrates (Bellwood *et al.*, 2004; Barber & Bellwood, 2005; Alfaro *et al.*, 2007; Williams, 2007; Williams & Duda, 2008). Our data indicates distinctive southern (Papua New Guinea and Australia) and northern (Indonesia, Philippines) elements to the diversity of *Portieria* in the IMA, which are not always evolutionarily closely related. Possibly, these northern and southern biotas were integrated by movement of tectonic plate elements, in particular from Australia and the Philippines, over the last 50 million years, as has also been suggested for fish and invertebrate groups (Rosen & Smith, 1988; Santini & Winterbottom, 2002; Carpenter & Springer, 2005; Renema *et al.*, 2008). A phylogenetic separation of these northern and southern *Portieria* clades in the IMA, corresponding to Wallace's line, can be explained by the low dispersal resulting in a geological imprint outweighing dispersal.

Apart from the IMA, three other regions harbour relatively high diversity of *Portieria* species: the Western Indian Ocean, the Northwestern Pacific, and the Southwestern Pacific.

The diversity of *Portieria* species in the Western Indian Ocean likely resulted from a few long distance dispersal events from the Central Indo-Pacific, followed by diversification within the region. From there, species dispersed north- and southward, and speciated possibly along a temperature gradient or across temperature barriers in the Somali-Arabian region, and temperate southern Africa, respectively. This supports the Southwestern and Northwestern Indian Oceans as generators of biodiversity, as has been indicated for several marine animal groups, including brittle-stars (Hoareau *et al.*, 2013) and gastropods (Postaire *et al.*, 2014). Upwelling systems in the Northwestern Indian Ocean have been shown to create stark biogeographic delineations in marine species composition (Schils & Wilson, 2006; Burt *et al.*, 2011) and are a likely driver of speciation. The relatively few dispersal events from the Central Indo-Pacific to the Western Indian Ocean, and the apparent lack of dispersal back to the Central Indo-Pacific indicates a clear separation between the two biogeographic

492 regions. This separation between Indian Ocean clades and the Central Indo-Pacific clades is
493 concordant with the Mid-Indian Ocean biogeographical barrier, which is one of the strongest inferred
494 marine barriers based on phylogenetic and present-day biodiversity patterns of coral reef fishes
495 (Cowman & Bellwood, 2013a; Hodge & Bellwood, 2016), and Indo-Pacific corals (Keith *et al.*, 2013).

496 In contrast to the Western Indian Ocean, the Northwestern and Southwestern Pacific have a much
497 closer connection with the Central Indo-Pacific. The relatively high *Portieria* species diversity in
498 those two regions can be explained by repeated north- and southward dispersal from the Central Indo-
499 Pacific, followed by *in situ* diversification, which was more extensive in the Southwestern than in the
500 Northwestern Pacific. In addition, several dispersal events were inferred from the two regions back to
501 the Central Indo-Pacific. In some cases, these dispersal events were inferred between neighbouring
502 regions with similar sea surface temperature regimes, for example between the northern Philippines
503 and southern Taiwan. Our results are consistent with the biodiversity feedback model, in which
504 biodiversity hotspots act as both centres of speciation (exporters of species), and centres of
505 accumulation (importers of species) (Bowen *et al.*, 2013).

506 The origin of *Portieria* in tropical North Pacific islands, such as Hawaii, Guam and Micronesia
507 resulted from multiple founder speciation events, but in most cases the source regions could not be
508 deduced with certainty. The sampled islands in this region, however, are geologically relatively young
509 and past paleogeographic patterns of small islands are not available. So, what might appear to be a
510 result of long-distance dispersal, could be a result of incremental short-distance dispersal where
511 intermediate areas have vanished throughout the course of *Portieria* evolution. Although species in
512 Hawaii and Guam showed a high haplotype diversity with a certain degree of population genetic
513 structuring, *in situ* diversification was limited on these islands, nor was there any dispersal from
514 Pacific islands back to the Central Indo-Pacific. This contrasts with studies on reef fishes where the
515 Hawaiian Archipelago has been shown to both produce and export new species (Eble *et al.*, 2011;
516 Bowen *et al.*, 2013).

517 In conclusion, our analyses contribute to a better understanding of the processes that produced
518 biodiversity patterns in the (sub)tropical Indo-Pacific. Although several groups of tropical marine
519 organisms exhibit congruent patterns of biodiversity, with a prominent hotspot in the IMA, there is no
520 single explanation for this pattern. Given the age and complex geological history of the IMA, along
521 with the vast diversity of organisms with different traits (e.g. dispersal capacity), multiple processes
522 have likely been at work (Barber, 2009; Halas & Winterbottom, 2009). Our phylogenetic analysis of
523 *Portieria* in the Indo-Pacific reflects the long and complex evolutionary history of this seaweed genus
524 and suggests that the observed biogeographical patterns are a combination of long-term persistence of
525 ancient lineages within confined geographical regions, including the IMA, and occasional long-
526 distance dispersal events.

The IMA biodiversity hotspot has provided a focus for numerous evolutionary and ecological studies, which have supported strategies for conservation efforts (Carpenter *et al.*, 2008). Our study adds to the growing body of evidence that the present-day species richness within the IMA hotspot results from a diverse range of evolutionary histories. As with many other groups of marine organisms, the IMA serves as both a species pump and a cradle of biodiversity of *Portieria* species, harbouring ancient lineages that were formed prior to the geological formation of the coral triangle and continue to produce species. Ecological and conservation related research also depends on a clear understanding of species boundaries, which is often problematic due to the prevalence of cryptic species in marine environments (Bickford *et al.*, 2007). This study shows once more that misconceptions about species boundaries may impact on our understanding of distributions and diversification of tropical seaweeds.

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BIOSKETCH

Frederik Leliaert is broadly interested in diversity, biogeography and evolution of algae. The research team consists of phycologists which are interested in seaweed diversity and the evolutionary processes generating marine biodiversity.

Author contributions: F.L., D.A.P., H.V., O.D.C conceived the study. F.L., D.A.P, C.F.D.G., T.S., S.G.A.D., G.W.S., M.K., A.R.S., S.-M.L., J.M.H., L.L.G., R.J.A., J.J.B., L.M., M.Z., C.V., E.C., H.V., O.D.C. conducted sampling. D.A.P, C.F.D.G., G.W.S., A.R.S., S.D., generated DNA sequence data. F.L., H.V. analysed the data. F.L. wrote the paper; and all authors commented on the final draft.

Figure legends

Figure 1. Geographical pattern of *Portieria* species richness. Species numbers in each of the 12 geographical regions are colour-coded, and summarized in the table below the map.

Figure 2. Latitudinal and longitudinal ranges of the 92 *Portieria* species. Colours indicate geographic region of the species. Species with latitudinal and/or longitudinal range larger than 500 km are labelled. Of these, only seven species had a latitudinal and/or longitudinal range larger than 2,000 km. Sp. 34 has a latitudinal and longitudinal range > 5,000 km, and occurs along the east African coast from South Africa to Oman, as well as in Madagascar and Sri Lanka.

Figure 3. Historical biogeographical reconstruction of the genus *Portieria*. The time calibrated phylogeny was inferred from the concatenated alignment (*cox2-3* spacer, *cox1*, *psbA*, *rbcL*, *rbcL-rbcS* spacer, and *EF2*) using 3 data partitions (see Materials and Methods). Asterisks (*) indicate Bayesian posterior probabilities > 0.95 and/or ML bootstrap values > 80% (the tree with divergence time confidence intervals, and branch support is shown in Fig. S5 in Appendix 4). Boxes at the tips indicate geographic ranges of extant *Portieria* species. Ancestral ranges, estimated under a DEC+J model, are indicated on the nodes as pie diagrams, and branch colours indicate ancestral ranges with likelihood >0.5 (grey branches indicate uncertain ancestral ranges). The map shows the 12 provinces used in the analysis. Species with letter codes (e.g., B21, S39 and V1ABC) were delimited by Payo *et al.* (2013), species numbers (e.g., sp.25, sp.58, sp. 60) are delimited in this study.

Figure 4. Frequency distributions of the counts of different kinds of events found in each of the 50 biogeographic stochastic mappings (BSMs) (province-level analysis) on the time calibrated phylogeny

(Fig. 3) under a DEC+J model. The x-axis gives the number of events in each of 50 BSMs; the y-axis gives the number of BSMs in which a specific number of events was observed.

Figure 5. Summary of biogeographical events for the Indo-Pacific genus *Portieria*. Number of events (narrow sympatry, founder events and anagenetic dispersal events) based on the results of the province-level historical biogeographical analysis (see Fig. S8). For clarity, the five inferred anagenetic dispersal events (BCD>BCD,B ; BD>BD,B; BF>F,BF; AB>AB,A and GJ->GJ,J) and the two inferred vicariance events (FJ>J,F and BF->F,B) are not indicated on the map.

APCAB201

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Oral Session

Topic A: Taxonomy, Biology, Ecology and Biodiversity of Algae

AO-01 **Initial isolation and optimization of culture medium of symbiotic microalgae (*Symbiodinium* sp.) with soft coral and sponges in some central coastal regions of Vietnam**
Dang Diem Hong, Pham Van Nhat, Hoang Thi Huong Quynh, Luu Thi Tam, Ngo Thi Hoai Thu, Le Thi Thom, Hoàng Thị Lan Anh, Nguyen Hoai Nam, Nguyen Thi Minh Hang and Chau Van Minh

AO-02 **Diversity, phylogeny, and distribution of *Hydroclathrus* (Scytosiphonaceae, Phaeophyceae) in the Pacific**
Wilfred John E. Santiañez, Erasmo C. Macaya, Kyung Min Lee, Sung Min Boo, Shinya Uwai, Akira Kurihara, Paul John L. Geraldino, Edna T. Ganzon-Fortes and Kazuhiro Kogame

AO-03 **Identification of high value ketocarotenoid-producing microalgae using simple spot test technique**
Peelada Cherdchukeattisak, Nipawan Puochsing, Preyanut Jareonsuk, Butsakorn Panmanee and Thanyanan Wannathong Brocklehurst

AO-04 **Molecular diversity of foliose Bangiales (Rhodophyta) in the Philippines**
Richard V. Dumilag, Zae-Zae A. Aguinaldo, Cynthia B. Mintu, Myrna P. Quinto, Wilberto D. Monotilla and Sandra L. Yap

AO-05 **Modern reassessment of the molecular phylogeny of siphonous green algae (Bryopsidales, Chlorophyta)**
Ma. Chiela M. Cremen, John West, Daryl Lam, Juan Lopez-Bautista and Heroen Verbruggen

AO-06 **The planktonic microalgae from Carmona River, Laguna Province: contribution to microalgae studies in the Philippines**
Emelina H. Mandia, Lawrence Victor Vitug, Renz Elmore Caguicla, Marc Nathaniel Gan and Bai Hannah Sapal

AO-07 **Phylogeography of the rhodophytes *Dichotomaria marginata* and *Gibsmithia hawaiiensis* in Southeast Asia**
Stefano G.A. Draisma, Daniela Gabriel, Shao-Lun Liu and Phaik-Eem Lim

AO-08 **Screening and optimization of marine and freshwater microalgae for high starch production**
Soopna A/P Puspanadan, Wong Xian Jin, Mohd Asyraf Bin Kassim and Lee Chee Keong

AO-09 **Macroalgal coverage, distribution and potential habitat mapping based on high resolution satellite data at Libukang Island, Malasoro Bay, Indonesia**
Nur A Setyawidati, Awaluddin Kaimiuddin, Ina P Wati, Helmi Muhammad, Ita Widowati, Pierre Olivier Liabot and Valérie Stiger-Pouvreau

AO-10 **Algal diversity of Indian Sunderbans with special reference to bioprospecting of potential strains**
Ruma Pal

• Pui Ling Tan AP1-03 Halymenia
• Yoon Yen Yow AP2-02 Gracilana cha
• Boghot Wichachutord Padin

AO-07

Phylogeography of the rhodophytes *Dichotomaria marginata* and *Gibsmithia hawaiiensis* in Southeast Asia

Stefano G.A. Draisma[1*], Daniela Gabriel[2], Shao-Lun Liu[3] and Phaik-Eem Lim[4]

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The Indo-Pacific transition zone is a marine biodiversity hotspot. Recent sea level fluctuations, present-day ocean currents, and organismal dispersal ability are believed to be responsible for the distribution of genetic lineages. Most molecular phylogeographic studies in the region have focused on marine animals. Very few macroalgal studies exist since the first study on seaweed phylogeography in the area was published in 2013. Studies in the brown algal genus *Sargassum* revealed genetic homogeneity across Southeast Asia in sharp contrast to most animal studies. However, *Sargassum* probably has a high dispersal ability aided by buoyancy providing pneumatocysts. Red algae are thought to be poor dispersers. A study of the red algal genus *Portieria* revealed that speciation in the marine realm can occur at spatial scales of less than 100 km. The present study explores the phylogeography of two subtidal red algae in the Indo-Malay archipelago, *i.e.*, circumtropical *Dichotomaria marginata* and Indo-West Pacific *Gibsmithia hawaiiensis*. Genetic connectivities between populations of both species are being studied using DNA sequences of, respectively, the plastid-encoded RuBisCO spacer and the mitochondrial *cox*2,3 spacer and (pseudo-)cryptic diversity was revealed in both taxa. Haplotype distributions of both *Gibsmithia* sp. and *D. marginata* indicate low genetic connectivity between basins.

Keywords: Cryptic diversity, Genetic connectivity, Haplotype diversity, Phylogeography, Rhodophyta

SAGE 2017

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Conference Program and Abstracts

Tuesday, August 29

Himalaya 1-4 Conference Room (6th floor)

Plenary Session

08.00	**Flying lizard phylogenomics and Sulawesi biogeography**	**Jimmy McGuire**
08.30	**Augmenting the PAIC Paradigm: new ways to study evolutionary processes of diversification in a model island archipelago**	**Rafe Brown**

Patterns of Biodiversity Symposium

09.00	Phylogeography of the Oriental Dwarf Kingfisher (*Ceyx erithaca*) using RADseq markers	Robert Moyle
09.15	From Hill rats (*Bunomys*) to Brotherly rats (*Frateromys*): the geography of diversification among rodents on Sulawesi, Indonesia	Heru Handika*
09.30	Improving geographic range estimates for island endemics	Susan M. Tsang
09.45	The diversity of illegally hunted wildlife from Rawa Aopa Watumohai National Park, Indonesia	Ikeu Sri Rejeki*
10.00	TEA & COFFEE BREAK	
10.30	From specimens to digital reference collections: A rapid and large-scale initiative to discover and present Singapore's biodiversity online	Yuchen Ang
10.45	Reinstatement and revision of the Philippine endemic genus *Adelmeria* Ridl. (Zingiberaceae)	Rudolph Valentino A.
11.00	What shapes tropical trees diversity? Specific and common factors driving diversity gradient in oaks	Damien D. Hinsinger
11.15	Taxonomy of foliose and fruticose lichens in Mt. Data, Mountain Province, Philippines	Shenly M. Gazo (L.C.)
11.30	Molecular phylogeny of pantropical *Panus* sensu stricto (Polyporaceae, Basidiomycota)	Jaya Seelan Sathiya Se
11.45	Molecular phylogeny of wild dimitic *Pleurotus* of Southeast Asia	Foo She Fui*
12.00	Phylogeography of two red algae in the Indo-Pacific transition zone	Stefano Draisma
12.15	LUNCH	

Plenary Session

13.15	**Biogeography of the mainland Southeast Asian tree snail genus *Amphidromus* and some genera of the ariophantid ground snails**	**Somsak Panha**

Patterns of Biodiversity Symposium

13.45	Accelerating the diet profile generation of assassin bugs (Heteroptera: Reduviidae) with next-generation sequencing of DNA meta-barcodes	Wei Song Hwang
14.00	Assignment of developmental stages of genus *Sparsorythus* (Insecta, Ephemeroptera, Tricorythidae) using mtDNA sequences	Jhoana M. Garces*
14.15	Very common, but generally neglected: minute marsh-loving beetles (Coleoptera: Limnichidae) of the Philippines – An update of diversity and distribution	Emmanuel D. Delocado*
14.30	*Aretidris*: a new genus of ants from the mountains of Luzon Island, Philippines	David Emmanuel Genera
14.45	First Anthicidae from Timor (Insecta: Coleoptera), and biogeographic patterns of the Papuan Anthicidae	Dmitry Telnov
15.00	Phylogeny and island biogeography of leaf beetle in the biodiversity hotspot of Sabah, Borneo	Kam-Cheng Yeong*
15.15	TEA & COFFEE BREAK	
15.45	Parade of little vampires: Strebilidae (Diptera: Hippoboscoidea) fauna of the Philippines	Ace Kevin Amarga
16.00	The biogeography of Australasian arachnids: what do we know?	Danilo Harms
16.15	Systematic analysis on scolopendromorph centipedes in Southeast Asia	Warut Siriwut
16.30	Philometrid Nematodes (Nematoda: Philometridae) from Balinese Waters	Kartika Dewi
16.45	Echinoderms of Maluku (Indonesia): An Inventory and Research History Review	Ana Setyastuti

Plenary Session

17.00 QUICKFIRE POSTER SESSION

18.00 POSTER SESSION (*Himalaya 5* Conference Room, 6th floor)

*Student presentation

Phylogeography of two red algae in the Indo-Pacific transition zone

Stefano Draisma[1], Daniela Gabriel[2], Shao-Lun Liu[3] & Phaik-Eem Lim[4]

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The Indo-Pacific transition zone is a marine biodiversity hotspot. Recent sea level fluctuations, present-day ocean currents, and organismal dispersal ability are believed to be responsible for the distribution of genetic lineages. Most phylogeographic studies in the region focused on animals. *Sargassum* was the first seaweed genus used in a phylogeographic study in the area (published in 2013). *Sargassum* showed genetic homogeneity across Southeast Asia in sharp contrast to most animal studies, possibly due to a high dispersal ability aided by buoyancy providing pneumatocysts. Red algae are thought to be poor dispersers. The present study explores the phylogeography of two subtidal reef algae in the Indo-Malay archipelago, *i.e.*, circumtropical *Dichotomaria marginata* (J. Ellis & Solander) Lamarck and (sub)tropical Indo-West Pacific *Gibsmithia hawaiiensis* Doty. DNA data reveal cryptic diversity in both taxa. *G. hawaiiensis* was not found, but four undescribed *Gibsmithia* species instead. DNA data demonstrate that *D. marginata* is indeed cosmopolitan, but also revealed an undescribed species. Haplotype distributions of both *Gibsmithia* and *Dichotomaria* indicate limited genetic connectivity between seas.

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Edited by Małgorzata Bąk, Przemysław Dąbek, Andrzej Witkowski

14:10-14:30	**Phylogeographic patterns indicate cryptic species diversity in the red alga *Gloiopeltis furcata* (Gigartinales) in the Northern Pacific region** Mi Yeon Yang et al.	**The tough and tenacious: thriving in natural analogues of warmer and acidic tropical marine waters** Michael Roleda et al.	**Predator control for large-scale cultivation using pulsed electric field technology** Thomas Dempster	**Conspecificity of an invasive ecotype of *Ulva compressa* from the SW Baltic with the model organism *Ulva mutabilis*** Sophie Steinhagen et al.
14:30-14:50	***The best is yet to come: molecular systematics of* Gibsmithia *(Dumontiaceae, Rhodophyta)*** Daniela Gabriel et al.	**Historical morphological changes of three species of Gelidiales** Beatriz Alfonso et al.	**Engineering acetic acid toxicity to grow mixotrophic microalgae at scale** Eneko Ganuza et al.	**Use of molecular methods in research on marine benthic diatoms and its benefits for biodiversity and environmental reconstructions of the coastal zone of the Baltic Sea and beyond** Chunlian Li et al.
				CS07: Physiology and biochemistry
14:50-15:10	**Multigene, organellar and nuclear genome data provide the big picture of red algal evolution** Su Yoon Hwan & Jun Mo Lee	**Linking physiological and transcriptomic analyses in *Desmarestia anceps* under future abiotic conditions** Sandra Heinrich et al.	**Balancing photosynthesis and respiration increases microalgal biomass productivity during photoheterotrophy on glycerol** Samir B. Grama et al.	**Needles in watery haystacks: finding novel molecules inducing diatom biofilm formation, excreted by bacteria** Lachlan Dow et al.
15:10-15:30	**Non-monophyly of *Bostrychia simpliciuscula* (Ceramiales, Rhodophyta): multiple species with very similar morphology; a revised taxonomy of cryptic species** Giuseppe Zuccarello et al.	**Role of phytoplankton metabolism down-regulation in a high CO_2 world** Cristina Sobrino et al.	**Engineering a heat-tolerant and ectoin-producing microalga in one strike** Kirsten Heimann et al.	**Interspecific variation in metabolic response to P limitation helps explain sympatric congeneric species diversity (in the northern Adriatic Sea)** Nataša Kužat et al.
15:30-15:50	***Evolutionary history and diversity of Mediterranean coralline algae: how much do we know?*** Fabio Rindi et al.	**Living in the boundary layer of kelp blades: refuge from ocean acidification or training for harsh conditions?** Fanny Noisette & C.L. Hurd	**You can't get me – developing *Haematococcus* strains resistant to *Paraphysoderma* infection** Noreen Hiegle et al.	**Heat stress memory is responsible for acquisition of thermotolerance in the red seaweed *Bangia fuscopurpurea*** Koji Mikami & I. Kishimoto
15:50-16:20	**Coffee Break**			