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REPORT

Taxonomy and life history of the *Acropora*-eating flatworm *Amakusaplana acroporae* nov. sp. (Polycladida: Prosthiostomidae)

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Abstract Efforts to culture and conserve acroporid corals in aquaria have led to the discovery of a corallivorous polyclad flatworm (known as AEFW – *Acropora*-eating flatworm), which, if not removed, can eat entire colonies. Live observations of the AEFW, whole mounts, serial histological sections and comparison of 28S rDNA sequences with other polyclads reveal that this is a new species belonging to the family Prosthiostomidae Lang, 1884 and previously monospecific genus *Amakusaplana* (Kato 1938). *Amakusaplana acroporae* is distinguished from *Amakusaplana ohshimai* by a different arrangement and number of eyes, a large seminal vesicle and dorso-ventrally compressed shell gland pouch. Typical of the genus, *A. acroporae*, lacks a ventral sucker and has a small notch at the midline of the anterior margin. Nematocysts and a *Symbiodinium* sp. of dinoflagellate from the coral are abundantly distributed in the gut and parenchyma. Individual adults lay multiple egg batches on the coral skeleton, each egg batch has 20–26 egg capsules, and each capsule contains between 3–7 embryos. Embryonic development takes approximately 21 days, during which time characteristics of a pelagic life stage (lobes and ciliary tufts) develop but are lost before hatching. The hatchling is capable of swimming but settles to the benthos quickly, and no zooxanthellae were observed in the animal at this stage. We suggest that intracapsular metamorphosis limits the dispersal potential of hatchlings and promotes recruitment of offspring into the natal habitat. The evolutionary and ecological significance of retaining lobes and ciliary tufts in the embryo are discussed. Camouflage, high fecundity and possible dispersal dimorphisms probably explain how *Amakusaplana acroporae* can cause *Acropora* sp. mortality in aquaria where natural predators may be absent.

Keywords Coral predator · Acropora-eating flatworm · Polyclad · Amakusaplana acroporae · Intracapsular larva · 28S rDNA phylogeny

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Introduction

The interactions of polyclad flatworms (Platyhelminthes) and their prey often come to light when the prey is of interest to humans. For example, predation of the bivalves *Crassostrea rhizophorae* and *Mytilus galloprovincialis* by *Stylochus (Stylochus) frontalis* and *Stylochus mediterraneus*, respectively, leads to increased mortality rates in these commercially important species (Galleni et al. 1980;

Littlewood and Marsbe 1990). Similarly, coral biologists, aquarists and conservationists are concerned by an *Acropora*-eating flatworm (commonly referred to as the "AEFW"). The AEFW is found on several species of *Acropora* in aquaria (so far reported or observed on *Acropora valida*, *A. pulchra, A. millepora*, *A. tortuosa, A. nana, A. tenuis, A. formosa, A. echinata* and *A. yongei*—Nosratpour 2008; R. Billings pers. obs.) and, if unchecked, its corallivory can lead to the death of entire colonies (Nosratpour 2008). Based on external morphology, the AEFW was identified as a Platyhelminthe of the order Polycladida and was tentatively assigned to the acotylean genus *Apidioplana* (Nosratpour 2008). However, to date, a detailed morphological, histological and molecular analysis of AEFW taxonomic affinity has been lacking. Whether the AEFW is as destructive an acroporid predator in the wild as it is in aquaria is not known. However, given the threatened or vulnerable status of many of the AEFW-affected acroporids (IUCN 2010) – and the importance of aquarium-reared acroporids to the sustainable hobby trade, education and reef restoration efforts (Yates and Carlson 1993; Borneman and Lowrie 2001; Carlson 1999) – a careful study of the AEFW's phylogenetic affinity and natural history is urgently needed.

Polyclad flatworms prey on a variety of marine invertebrates, including molluscs (Pearse and Wharton 1938; Littlewood and Marsbe 1990; Ritson-Williams et al. 2006), urochordates (Crozier 1917; Millar 1971; Newman and Cannon 1994; Baeza et al. 1997; Newman et al. 2000), crustaceans (Murina et al. 1995) and cnidarians (Kawaguti 1944; Jokiel and Townsley 1974; Poulter 1975). While some show prey preference, e.g. *Maritigrella crozieri* (Crozier 1917) and *Prostheceraeus roseus* (Perez-Portela and Turon 2007), little is known about prey specificity. Several polyclad species have been found living in various degrees of association with cnidarians (Table 1), but predation has been inferred in only a few instances by the presence of cnidae (nematocysts, spirocysts and ptychocysts) in the epithelium and/or gut (Bock 1922; Karling 1966; Poulter 1975; Holleman 1998).

Knowledge of the life history strategies of polyclads is the key to understanding their population dynamics and therefore may also be useful in managing their predatory impact. Polyclads are the only free-living platyhelminth clade in which members exhibit a gradient of developmental modes, from 'direct' development (i.e. embryos hatching as a benthic juvenile) through 'intermediate' development (i.e. intracapsular larva – larva with lobes and ciliary band retained within an egg case, and hatching as a benthic juvenile – Kato 1940) to 'indirect' development (i.e. with a planktonic life history stage with lobes and ciliary band, e.g. Götte's and Müller's larvae). Indirect development has been described in both suborders of polyclads – the Cotylea and Acotylea – while intracapsular larva and direct development have, until now, been found exclusively within the Acotylea (Smith et al. 2002).

The aims of this study were to identify the AEFW species using morphological, histological and molecular

Table 1 Associations of polyclads with cnidarian taxa

Polyclad species	Cnidarian taxa (Order)	Association	Reference	Notes
Apidioplana mira	*Melitodes* sp. (Alcyonacea)	?	Bock (1926)	
Apidioplana okadai	*Melithaea flabellifera* (Alcyonacea)	?	?	
Unknown planarians	*Montipora* sp. (Scleractinia)	?	Kawaguti (1944)	
	Lobophyllia sp.			
	Stylophora sp.			
	Hydroplana sp.			
Prosthiostomum (P.) montiporae	*Montipora verrucusa* (Scleractinia)	Obligate ectoparasite symbiont	Jokiel and Townsley (1974)	No cnidae observed in polyclad gut (Poulter, 1975)
Stylochoplana tarda		?	Karling (1966)	Nematocysts in gut
Stylochoplana inquilina	*Calliactis armillatus*, (Actiniaria)	Predator	Poulter (1975)	Nematocysts in gut
Anonymus virilis	?	Predator	Karling (1966)	Nematocysts in gut
Anonymus multivirilis	?	Predator	Holleman (1998)	Nematocysts in gut and dorsal epidermis
Anonymus kaikourensis	?	Predator	Holleman (1998)	Nematocysts in gut and dorsal epidermis
Chromoplana bella	Hydrozoa	Predator	Karling (1966)	Nematocysts in gut
Amyella lineata	Hydrozoa	Predator	Bock (1922)	Nematocysts in gut

characters and to collect the first data on AEFW development and life history, as a foundation for future predator management efforts. We have determined that the AEFW is a new species, belonging to the suborder Cotylea, the family Prosthiostomidae and the previously monospecific genus *Amakusaplana* (Kato 1938). This species exhibits a number of interesting morphological and life history conditions – including intra-capsular metamorphosis – that may represent adaptations to a corallivorous existence.

Materials and methods

Collection

Live observations of adult worms were made from specimens collected in 2009 from one of the author's (R. Billings) aquaria in Virginia, USA. Developmental data were gathered from observations of live embryos in egg capsules attached to the coral skeleton. For histological and whole mount analysis, adults were fixed on 4% frozen formaldehyde in sea water overnight at room temperature and were then rinsed in sea water multiple times before being transferred to 70% ethanol for storage. For molecular analysis, adult specimens from aquaria in Virginia and New York (Atlantis Marine World) were preserved in 95% undenatured ethanol. Embryos were manually extracted from egg capsules at different stages and were fixed, along with hatchlings, in 4% formaldehyde in 1X phosphate-buffered saline (PBS) for 20 min at room temperature. Specimens were then rinsed three times in 1XPBS and stored in 1XPBS and sodium azide at 4°C for phalloidin staining and immunohistochemistry.

Phalloidin staining and immunohistochemistry

Filamentous actin in pre-hatching and hatching stages was labelled with Alexa488 phalloidin (Molecular Probes) following the protocol of Rawlinson (2010). Epidermal cilia of pre-hatching and hatching stages were labelled with anti-tyrosinated tubulin (Sigma) diluted 1:500 in 1XPBS and 0.1% Triton x-100 (PBST) and were detected with a FITC-conjugated secondary antibody against mouse igM (Molecular probes) diluted 1:800 in PBST. Individuals were mounted in Vectashield antifade mounting media (Vector Laboratories, Burlingame, CA) and imaged using a Zeiss LSM 510 confocal laser scanning microscope. Digital images were assembled in Adobe Photoshop CS.

Histology and whole mounts

For histology, whole specimens of the AEFW were graded into 100% ethanol. Specimens were then cleared in Histoclear (National Diagnostics) for 24 h, infiltrated with 1:1 histoclear/paraffin for 24 h and equilibrated in molten paraffin for 24 h (all steps performed in a 60°C paraffin oven, with several changes at each step). Specimens were then embedded in fresh paraffin and left to harden at room temperature for 24 h prior to sectioning. Entire specimens were sectioned in the cross- or sagittal plane at 6 μM on a rotary microtome. Sections were mounted on glass slides and stained with Mayer's haematoxylin and eosin Y as follows: 2 × 5 min in histosol (National Diagnostics), 2 × 2 min in 100% ethanol, 2 min in 70% ethanol, 2 min in 30% ethanol, 2 min in distilled water, 15 min in Mayer's haematoxylin, 20 min in running tap water, 1 min in eosin Y, 2 × 2 min in 95% ethanol, 2 × 2 min in 100% ethanol and 2 × 5 min in histosol. From histosol, slides were coverslipped with DPX (BDH).

For whole mounts, specimens were graded from 70% ethanol into 100% ethanol and then cleared for 1 h in histosol at room temperature (with three changes). Specimens were then equilibrated in DPX, mounted and coverslipped. Specimens were imaged on a Zeiss Axioscope fluorescent compound microscope.

Generation of molecular tags and phylogenetic analysis

For all platyhelminth taxa sampled, total genomic DNA was extracted from a small piece of excised marginal tissue using the Qiagen DNeasy Blood and Tissue kit. Using genomic DNA as a template, the D1–D2 region of the 28S rDNA gene was amplified using the universal FW1 and REV1 primer sequences of Sonnenberg et al. (2007). PCR amplification using universal primers with AEFW genomic DNA repeatedly amplified a fragment of the 28S rDNA locus of the dinoflagellate *Symbiodinium* sp. (Genbank accession number HQ678179), so a novel forward (3′–5′) and reverse (3′–5′) primer pair were designed for this taxon, based on conserved regions within aligned polyclad 28S rDNA sequences. All PCRs were carried out using the following cycle temperatures/times: 4 min at 94°C; 45 cycles of 20 s at 94°C, 20 s at 52.5°C and 90 s at 72°C; 8 min at 72°C for a final extension. PCR was electrophoresed in a 1% agarose gel, and products (~920 bp) were excised and purified using the Qiagen MinElute Gel Extraction kit. Purified PCR products were then ligated into the pGemT-easy vector system and cloned with JM109 chemically competent *E. coli*. All plasmid minipreps were sequenced in both directions using T7 and SP6 primers. Accession numbers for all sequences are listed in Table 2.

Sequences were aligned and verified using the ClustalW algorithm in MacVector. Phylogenetic trees were constructed using maximum likelihood (ML) methods in Paup* 4.0b10 (Swofford 2002) and Bayesian Inference (BI) in MrBayes 3.2 (Ronquist and Huelsenbeck 2003). For

Table 2 Platyhelminth taxa included in phylogenetic analysis of 28S rDNA sequences

Species	Collection site	Genbank
Outgroup		
Proseriata		
Parotoplana renatae Ax, 1956	?	AJ270176(Littlewood et al. 2000)
Macrostomida		
Macrostomum lignano Ladurner, Schärer, Salvenmoser, & Rieger, 2005	in culture, Innsbruck	HQ659019
Ingroup		
Polycladida		
Imogine oculifera Girard, 1853	Fort Pierce, Fl, USA	HQ659007
Notoplana australis (Schmarda, 1859)	Phillip Island, Australia	HQ659015
Melloplana ferruginea (Schmarda, 1859)	Tavernier Key, Fl, USA	HQ659014
Cestoplana rubrocincta (Grube) Lang 1884	Phillip Island, Australia	HQ659009
Echinoplana celerrima Haswell, 1907	Phillip Island, Australia	HQ659020
Idioplana australiensis Woodworth, 1898	Phillip Island, Australia	HQ659008
Pericelis cata Marcus & Marcus, 1968	?	EU679114 (Litvaitis & Bolaños *unpub)*
Prosthiostomum siphunculus (Delle Chiaje, 1822)	Mataró, Spain	HQ659012
Amakusaplana acroporae	(i) Personal aquarium (R. Billings) Virginia	HQ659011
	(ii) Atlantis Marine World, Long Island, NY, USA	HQ659010
Maritigrella crozieri (Hyman 1939)	Long Key, Fl, USA	HQ659013
Pseudobiceros splendidus (Lang 1884)	Fort Pierce, Fl, USA	HQ659016
Thysanozoon brocchii (Risso 1818)	Phillip Island, Australia	HQ659017
Yungia sp. Lang 1884	Fort Pierce, Fl, USA	HQ659018

ML analysis, ModelTest Server (Posada 2006) was used to select the most appropriate model of nucleotide substitution. The TrN + G and GTR + I+G models were selected based on the hierarchical likelihood ratio test and the Akaike Information Criterion, respectively, and ML analysis conducted under both models produced identical tree topologies. Node support for the ML tree was determined by bootstrapping (with 100 replicates). For BI, analysis was performed for 2,000,000 generations with a sampling frequency of 100. Node support for BI was determined by posterior probabilities.

Results

Systematics

Order: Polycladida Lang (1884)
Sub-order: Cotylea Lang (1884)
Super-family: Euryleptoidea Faubel (1984)
Family: Prosthiostomidae Lang (1884)
Genus: *Amakusaplana* Kato (1938)
Amakusaplana acroporae nov. sp.

Material examined

Morphological examination of 7 mature specimens from the aquaria in Virginia and from Atlantis Marine World (Long Island, NY) and 30 early life history stages, including embryos and hatchlings from Virginia, was carried out.

Type material

Holotype–adult worm: whole mount (Natural History Museum, London, UK, accession number: 2010.9.27.1).

Paratypes

(a) sagittal sections of adult (Natural History Museum, London, UK, accession number: 2010.9.27.2)
(b) Cross-sections of adult (Natural History Museum, London, UK, accession number: 2010.9.27.3)
(c) sagittal sections of adult (National Museum of Natural History, Washington DC, USA USNM1153932).

Type repository Natural History Museum, London, UK

Type locality In personal aquarium of R. Billings, Virginia, USA, found on *Acropora valida, A. tortuosa,*

A. nana, A. tenuis, A. formosa, A. echinata, A. millepora and *A. yongei*.

Other material observed

(a) sagittal sections of one adult
(b) one specimen from Atlantis Marine World for DNA (Genbank Accession: HQ659010).
(c) one specimen from Virginia aquarium for DNA (Genbank Accessions: HQ659011).
(d) specimens from Atlantis Marine World
(e) 10 embryos and 20 hatchlings

Comparative material examined

Unfortunately, attempts to locate Kato's type material of *Amakusaplana ohshimai* collected from around Amakusa Marine Biological Laboratory, Japan, were unsuccessful. Our data were therefore compared to the species description (Kato 1938).

Etymology

The name indicates its close association with *Acropora* species, on which it feeds and lays its eggs.

Synonym

Commonly referred to as the *Acropora*-eating flatworm (AEFW).

Distribution

To the authors' knowledge, this polyclad has only been collected from aquaria to date. The specimens examined here were collected from aquaria in the United States (Atlantis Marine World, Long Island, New York and a private Virginia aquarium). The Birch aquarium at Scripps has reported identical polyclads (Nosratpour 2008), and there are anecdotal reports of a similar flatworm occurring in aquaria in Germany and the UK, though whether these are, in fact, the same remains to be determined. Distribution in the wild is unknown. However, it is found on Indo-Pacific species of *Acropora*.

Diagnosis

The genus *Amakusaplana* is a member of the Prosthiostomidae, exhibiting the following characteristics of the family: absence of tentacles, a mouth at the anterior end of pharyngeal chamber, a tubular pharynx, a large muscular seminal vesicle adjacent to a pair of thick-walled accessory vesicles, a penis papilla and stylet enclosed in a penis-pocket, a short vagina that is looped anteriorly and uterine canals arranged in an H-shaped figure (Prudhoe 1985). *Amakusaplana* may be distinguished from other prosthiostomid genera by the absence of a ventral sucker, the presence of a slight median depression in the anterior margin and irregularly scattered eyes in the anterior region of the body (Kato 1938). The type and only other species of *Amakusaplana* described is *Amakusaplana ohshimai* (Kato 1938).

Amakusaplana acroporae (Fig. 1) is distinguished from *Amakusaplana ohshimai* Kato 1938 by differences in number and distribution of eyes around the anterior end of the worm (Fig. 2a). Mature *A. acroporae* have 32 ± 3 (mean $\pm$ SD; $n = 5$) cerebral eyes subepidermally radiating out from the brain, with few distributed around the anterior sixth of the pharynx. Four to five marginal eyes are present, two either side of the depression on the frontal margin. *A. ohshimai* has approximately 94 eyes scattered around the anterior end of the body. These are hardly distinguishable into marginal, cerebral and frontal groups, and they extend along either side of the anterior half of the pharynx (Kato 1938).

A. *acroporae* has a large seminal vesicle – compared to the small vesicle found in *A. ohshimai* – but has a smaller male atrium than *A. ohshimai* (Fig. 2b). In *A. acroporae,* the large and bulbous female atrium is overlain by a dorsoventrally compressed shell gland pouch, whereas *A. ohshimai* has a small female atrium surrounded by a wide shell gland pouch. In mature *A. acroporae*, a distinct oval egg chamber connects the two uteri to the egg canal (or vagina interna) (Figs. 1d, 2b, 4b). This was not described in *A. ohshimai.* Kato (1938) states that *A. ohshimai* specimens were collected from Madreporarian (i.e. scleractinians or stony) corals, but does not resolve the coral identification any further.

A. acroporae resembles *Prosthiostomum (P.) montiporae* with respect to pharynx length and shape (short, cleft and scroll-like – see "Description") and the presence of an ova-filled chamber between the two uteri that joins the vagina. *A. acroporae* is distinguished from *P. (P.) montiporae* on the basis of uteri that do not join posteriorly under the main intestine, the absence of a ventral sucker, general body shape, eye arrangement and sexual apparatus arrangement.

Description

External features: The adults examined ranged in size from 6–17 mm long to 3–10 mm wide, and all were sexually mature. On the coral, the worms are oval in shape, concave and fleshy. The worms showed a brown reticulate pattern of coloration on a white background, and this coloration is

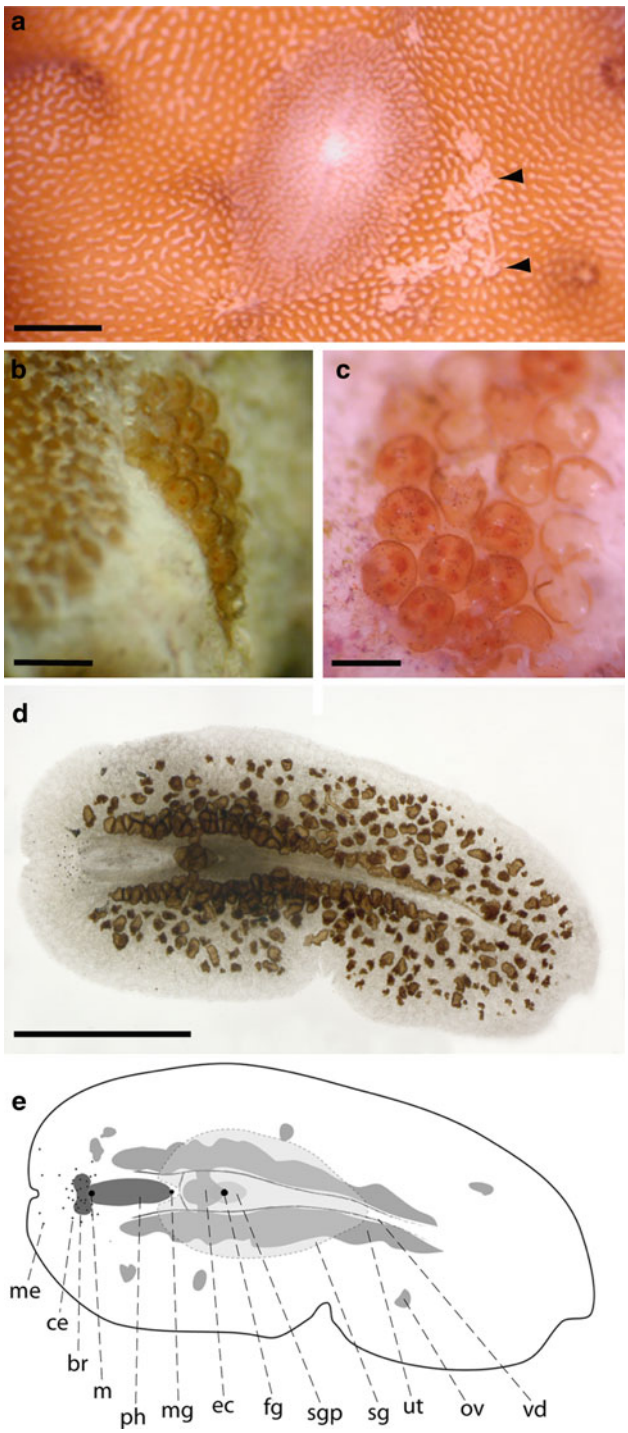

Fig. 1 *Amakusaplana acroporae* sp. nov. **a** Live adult on *Acropora* sp. with cluster of feeding scars to right (*arrowheads*) (scale = 5 mm). **b** Egg batch attached to the coral skeleton next to live coral tissue (scale = 2 mm). **c** Egg batch with some hatched capsules and others containing between 3 and 7 embryos (scale = 1 mm). **d** Cleared whole mount and e. schematic representation showing *A. acroporae* gross morphology (scale = 5 mm). *br* brain, *ce* cerebral eye, *ec* egg chamber, *fg* female gonopore, *m* mouth, *me* marginal eye, *mg* male gonopore, *ov* ovary, *ph* pharynx, *sg* shell glands, *sgp* shell gland pouch, *ut* uteri, *vd* vas deferens

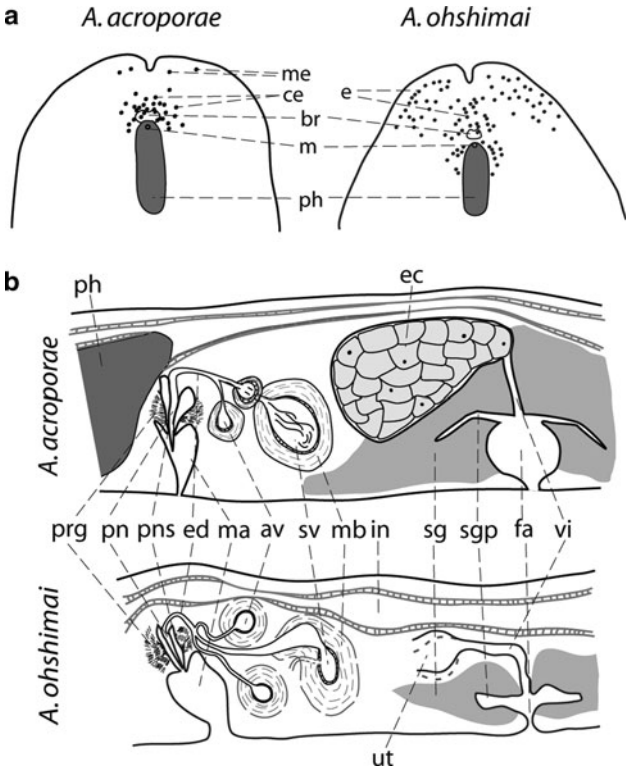

Fig. 2 Diagrammatic representation of *Amakusaplana acroporae* morphology and comparison with *A. ohshimai* (from Kato, 1939), showing **a** the distribution of eyes around the anterior end and **b** a sagittal view of the male and female reproductive systems. *av* accessory vesicle, *br* brain, *ce* cerebral eye, *e* eyes, *ec* egg chamber, *ed* ejaculatory duct, *fa* female atrium, *in* intestine, *m* mouth, *ma* male atrium, *me* marginal eyes, *ph* pharynx, *pn* penis, *pns* penis sheath, *prg* prostate glands, *sg* shell glands, *sgp* shell gland pouch, *sv* seminal vesicle, *ut* uteri, *vi vagina interna*

due to coral tissue and zooxanthellae inside the gut and parenchyma. Accordingly, the polyclads camouflage very effectively against the *Acropora* sp. and are found closely appressed to its external surface (Fig. 1a). Feeding scars on the coral tissue (Fig. 1a) and egg batches on the coral skeleton (Fig. 1b, c) are generally the first indication that the worms are present. No tentacles of the nuchal or pseudo-tentacle type are present. Thirty-two cerebral eyes are scattered anterior to the pharynx, and 2–3 marginal eyes are located either side of an indentation situated medially on the anterior margin (Fig. 2a). A ventral sucker is absent.

Body wall: The epidermis is fully ciliated. Rhabdites are abundant in the dorsal epidermis (Fig. 3a), but are absent from the ventral epidermis (Fig. 3b). Structures resembling nematocysts from *Acropora* sp. are also present in the dorsal epidermis (Figs. 3a, 5a).

Digestive system: The mouth is located behind the brain, slightly posterior to the anterior limit of the pharynx (Figs. 1e, 3d). The pharynx is barrel-shaped, tubular,

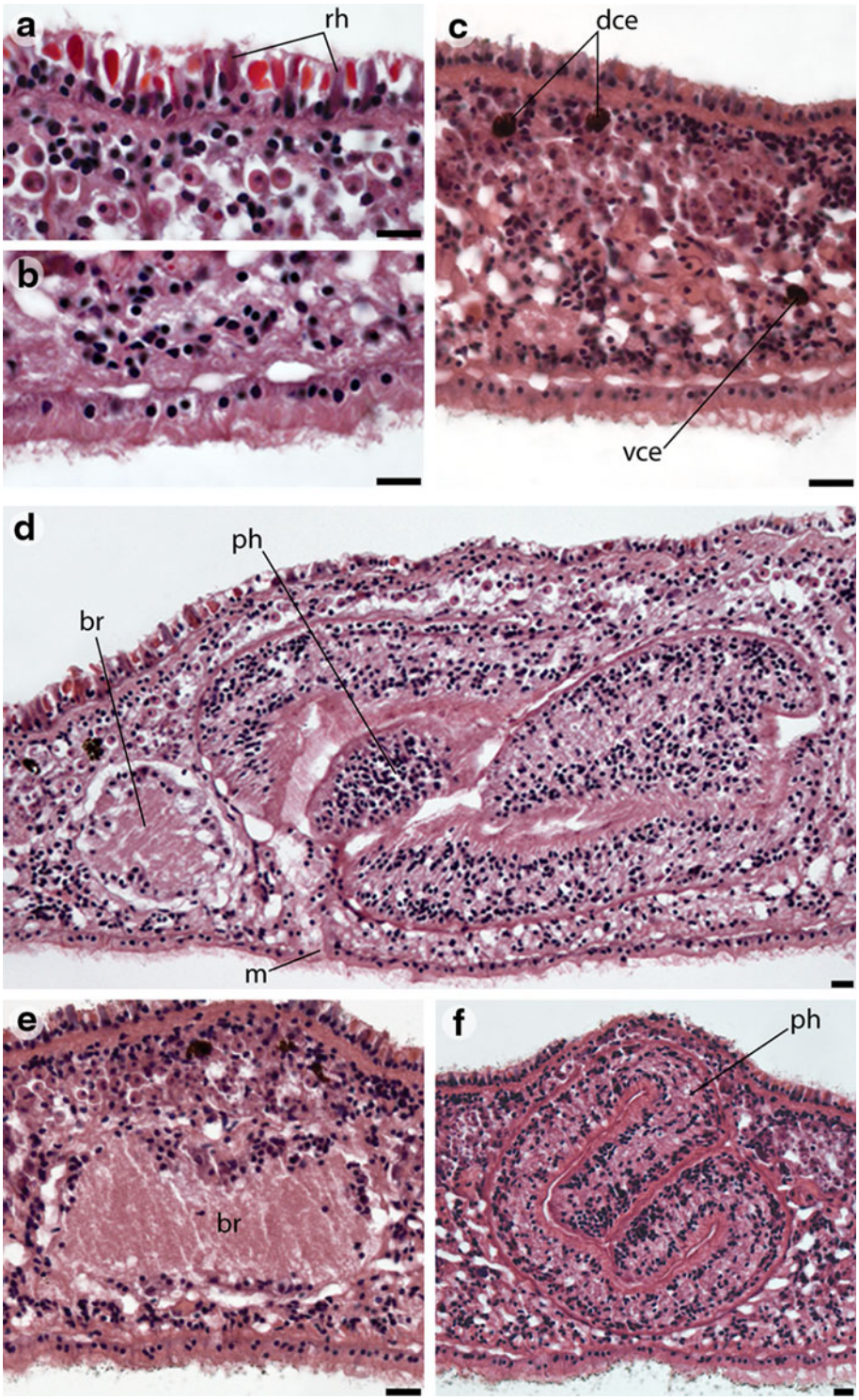

Fig. 3 Histological sections of *Amakusaplana acroporae* show **a** abundant rhabdites (*rh*) in the dorsal epidermis and **b** an absence of rhabdites in the ventral epidermis (sagittal views). Sections through the head reveal **c** subepidermal cerebral eyes both dorsally (*dce*) and ventrally (*vce*)(transverse view), **d** the position of the brain (*br*) immediately anterior to the mouth (*m*) and pharynx (*ph*)(sagittal view) and **e** the bilobed morphology and densely nucleated rind of the brain (*br*)(transverse view). **f** A transverse section through the pharynx (*ph*) reveals a cleft morphology. Scale = 10 μm

muscular and cleaved (Fig. 3d) and is 12–13% of the length of the body length. The pharynx appears scroll-like in cross-section, with the two ends having curled in on themselves (Fig. 3f). The anterior median branch of the intestine runs over the pharynx towards the cerebral region (Fig. 3d). Within the gut and parenchyma, zooxanthellae (*Symbiodinium* sp., based on 28S rDNA sequence – see "Material and Methods") are highly abundant (Fig. 5a). Their lipid bodies are visible (Fig. 5c), and their auto-fluorescence distinguishes them from polyclad cells

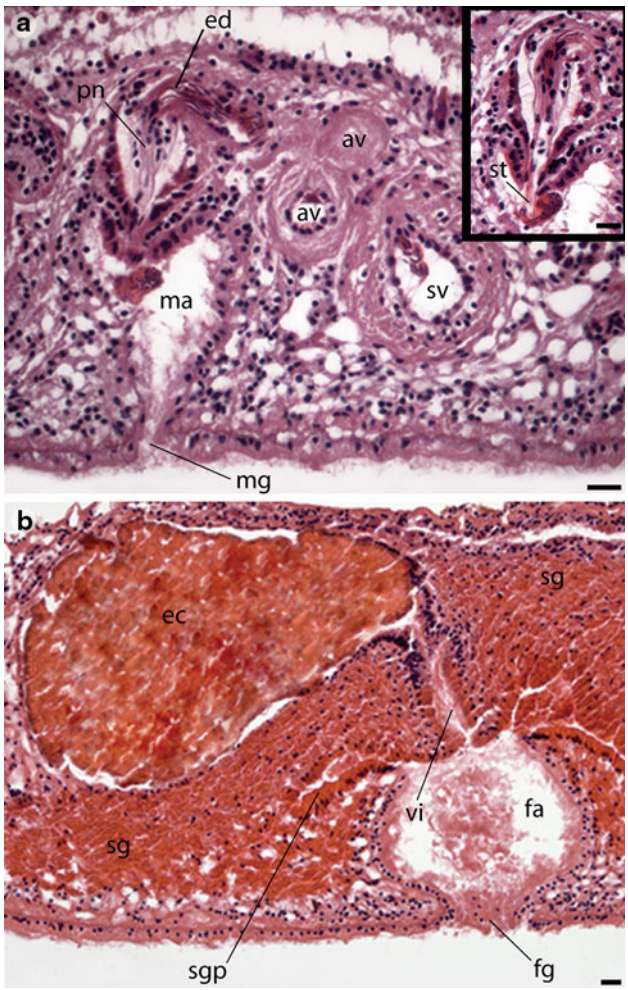

Fig. 4 **a** The male reproductive system consists of a penis (*pn*) with stylet (inset; *st*) protruding from the penis sheath into the male atrium (*ma*) dorsal to the male gonopore (*mg*), and a seminal (*sv*) and two accessory vesicles (*av*) connected via the ejaculatory duct (*ed*) to the penis (sagittal views). **b** A composite of three adjacent sagittal sections shows the female gonopore (*fg*) opening into the female atrium (*fa*), above which sit dorsoventrally flattened shell gland pouches (*sgp*) that are surrounded by extensive shell glands (*sg*), the *vagina interna* (*vi*) leads to the egg chamber (*ec*). Scale = 10 μm

(Fig. 5b). They are ~8 μm in diameter and distributed throughout the body, but are not observed intracellularly (Fig. 5c). Unfired nematocysts are present in the gut (Fig. 5d) and possibly also in the dorsal epidermis (Figs. 3a, 5a).

Eyes and brain: Anterior to the pharynx is a bilobed brain (Fig. 3d, e). The two lobe masses are connected by a central neuropile and are surrounded by a nucleated rind. Approximately 29 cerebral eyes are scattered subepidermally dorsal and anterior to the brain (Fig. 3c, d, e), and three ventral cerebral eyes are found subepidermally, anterior to the brain – two on the right and one on the left. Four or five marginal eyes are also present (Fig. 2a).

Reproductive anatomy: The male gonopore is located posterior to the pharynx and anterior to the female gonopore. The male system is directed posteriorly relative to the gonopore (Fig. 2b) and consists of a penis armed with long scleratized stylet (Fig. 4a inset. 30 μm long), which sits in the penis sheath and protrudes into the male atrium. The penis is connected via the ejaculatory duct to two accessory vesicles and a large seminal vesicle (Figs. 2b, 4a). The accessory vesicles and seminal vesicle are each bound by a muscular sheath. Prostatic glands empty into the penis sheath (Fig. 2b).

The female reproductive system is directed anteriorly relative to the gonopore (Figs. 2b, 4b) and consists of a large, bulbous atrium, on top of which sits a dorsoventrally compressed shell gland pouch. The shell glands extend from the posterior region of the pharynx into the posterior third of the animal. The egg canal (or *vagina interna*) extends dorsally from the female atrium to a large oval egg chamber (Figs. 1d, 2b, 4b). Connections extend bilaterally from the midline egg chamber to the paired uteri, which flank the pharynx and the main dorsal intestinal tract (Fig. 1d). The uteri do not join posteriorly under the main intestine, as is the case in *Prosthiostomum. (P.) montipora, P. (L.) matarazzoi* and *P. (L.) utarum*. Ovaries are scattered throughout the body (Fig. 1d).

Development: It is not known how many egg batches an adult will lay in its lifetime. All egg batches observed were found on bare coral skeleton as opposed to live tissue. The number of egg capsules per batch ranged from 20–26 (n = 10), and within each capsule, there were 3–7 embryos (capsule n = 15). The length of embryonic development is approximately 21 days at 76–78°F (~25°C).

Interestingly, embryos that were manually extracted from their egg capsules post-gastrulation (Fig. 6a, b) exhibited anatomical features typical of the pelagic life history ('larval') stage of an indirect developing species (i.e. muscular lobes with longer cilia at their distal margins, as indicated by phalloidin staining and tyrosinated tubulin immunoreactivity, respectively). Embryos possess eight short lobes – a dorsal lobe, an oral hood and three paired lateral lobes (the dorsolateral, lateral and ventrolateral lobes) – which can be seen by visualizing the body wall musculature scaffold with phalloidin (Fig. 6c). Tufts of longer cilia are associated with each lobe (Fig. 6d). Embryos have four cerebral eyes and one epidermal eye (Fig. 6b). No zooxanthellae were present at this developmental stage, indicating that there is no transfer of dinoflagellates from parent to offspring in the oocyte.

At hatching (Fig. 6e, f), juveniles emerge resembling small-scale adults, and lobes and ciliary tufts are no longer present (presumably having been resorbed or lost – Fig. 6g, h). Hatchlings are dorsoventrally flattened and range in size from 250–300 μm in length and 110–130 μm in width. The

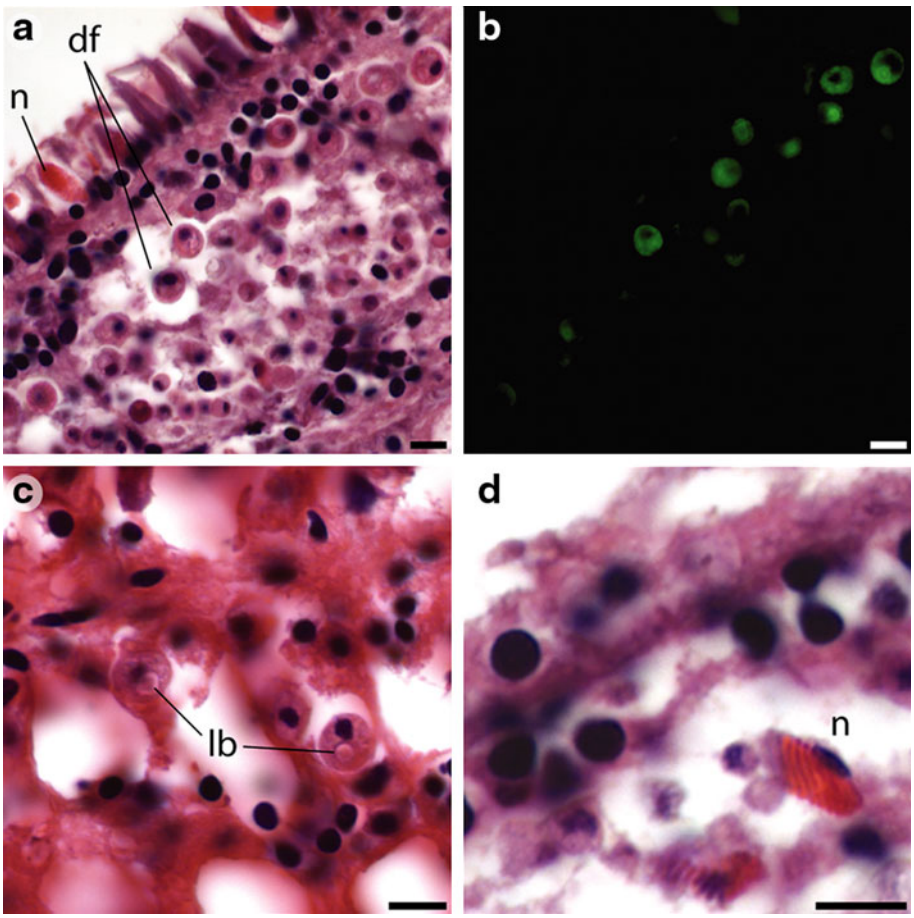

Fig. 5 **a** *Symbiodinium* sp. of dinoflagellate (*df*) are distributed abundantly throughout the gut and parenchyma of *Amukusaplana acroporae.* These dinoflagellates exhibit a distinct cell morphology and **b** autofluoresce (Section 5a under fluorescent light). **c** The lipid body (*lb*) of the *Symbiodinum* sp. is evident under higher magnification. These zooxanthellae are not intracellular. **d** Unfired nematocysts (*n*) from *Acropora* sp. are also present in the gut and parenchyma, and possibly in the dorsal epidermis (see 5a). Scale = 5 μm

number of eyes was on average 9; 8 clustered around the brain and another situated more anteriorly in the epidermis (Fig. 6e, f). Like the hatchlings of many 'direct' developing polyclads, *A. acroporae* hatchlings are able to swim into the water column, and this may be sufficient to transport individuals to neighbouring coral colonies. When kept in isolation, hatchlings would generally rest on the bottom of the dish. If kept with coral fragments, hatchlings would swim into the skeleton immediately. It is presumed that hatchlings are able to feed on coral tissue immediately, as zooxanthellae were seen in the gut of recent hatchlings.

Molecular relationships: The Bayesian Inference and maximum likelihood analyses of 28S rDNA sequence data (Fig. 7) resolve *Amakusaplana acroporae* to the suborder Cotylea, as the sister group to *Prosthiostomum siphunculus.* The BI and ML analyses gave trees of identical topology. These findings, therefore, independently confirm the higher-order (i.e. prosthiostomid) phylogenetic affinity of *A. acroporae* based on the morphology described above. The ingroup, Polycladida, is divided into two clades: one well-supported clade including *A. acroporae* with other cotylean species (Cotylea *sensu* Lang 1884) and a second less well-supported clade (BI: 96%, ML: < 50%) including *Pericelis cata* – conventionally classified as a cotylean – as the sister to the Acotylea (*sensu* Lang 1884).

Two specimens of *A. acroporae* from different aquaria (Virginia and New York) resolve as well-supported sister taxa, with a pairwise genetic distance of ~0.003. This level of genetic divergence falls within the range of intraspecific variation observed in the D1-D2 LSU region of other polyclad taxa (e.g. *Pseudoceros bicolor*—Litvaitis et al. 2010). However, more extensive sampling of *Amakusaplana acroporae* specimens from different aquaria is needed to rigorously test for the possibility sub- or cryptic speciation within this group.

Discussion

For at least 10 years, the enigmatic AEFW has been a destructive predator of captive *Acropora* colonies. Until now, a proper taxonomic assessment of this animal has been lacking. Here, for the first time, we show that the AEFW is a polyclad belonging to the genus *Amakusaplana*, and we are designating this a new species, *Amakusaplana acroporae*, based on the morphological characters described above.

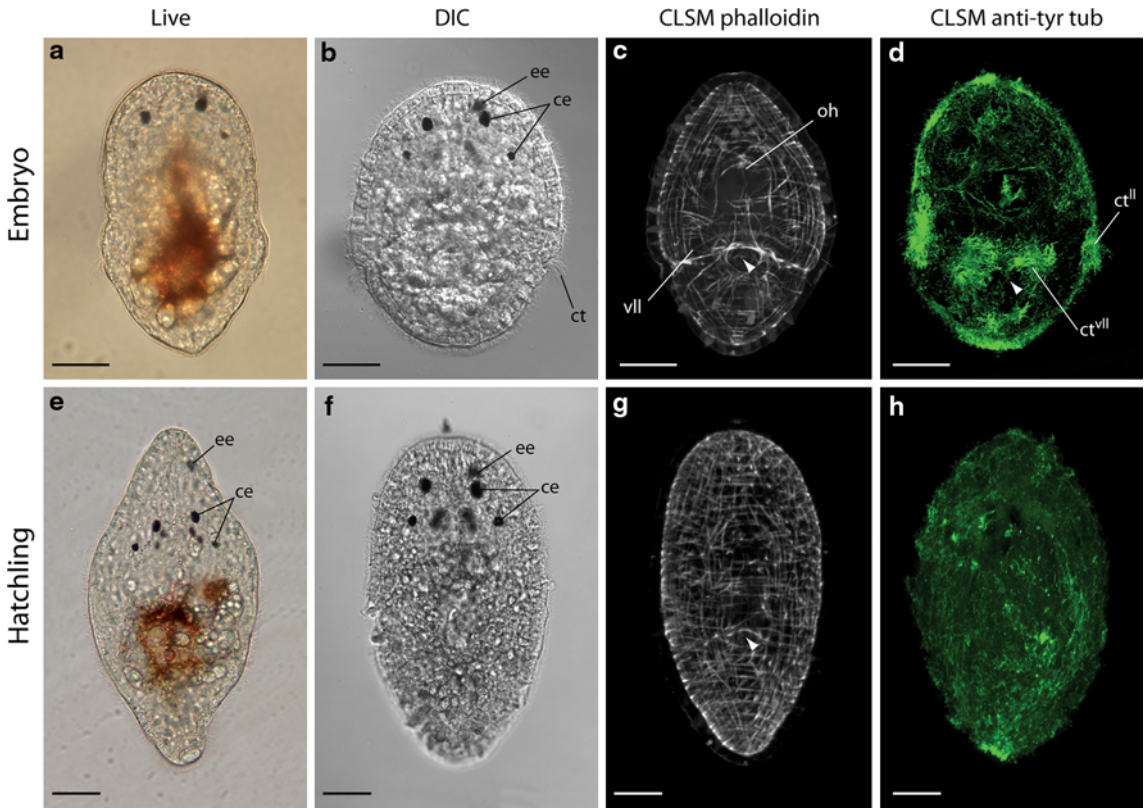

Fig. 6 **a** Live and **b** fixed pre-hatching embryos. **c** Phalloidin staining of F-actin reveals musculature associated with embryonic lobes. **d** Visualization of epidermal ciliation by anti-tyrosinated tubulin immunoreactivity reveals ciliary tufts on the distal margins of the lobes. **e** Live and **f** fixed hatchling. **g** At hatchling stage, lobes are no longer visible and **h** ciliary tufts are no longer distinguishable. All images in ventral view. *Scale bars* 50 μm. *ce* cerebral eyes, *ct* ciliary tuft, *ee* epidermal eye, ct^{II} ciliary tuft associated with lateral lobes; *oh* oral hood, ct^{vII} ciliary tuft associated with ventrolateral lobes; mouth (*arrowhead*). (DIC–differential interference contrast, CLSM–confocal laser scanning microscopy)

Taxonomic remarks and morphological considerations

Nosratpour (2008) tentatively assigned the AEFW to the Apidioplanidae, a monogeneric family in the suborder Acotylea. However, the morphological and histological analyses presented here demonstrate that the AEFW belongs to the suborder Cotylea, the family Prosthiostomidae and the genus *Amakusaplana*. Cotylean affinity is supported by a tubular pharynx, gonopores in the anterior half of the animal and an enlarged dorsoventrally compressed shell gland pouch, while the lack of tentacles, a cleft pharynx and two accessory vesicles in the male reproductive system are features shared with other prosthiostomid taxa. Finally, the AEFW lacks a ventral sucker, a condition that, among Prosthiostomidae, has only been described in the genus *Amakusaplana* (Kato 1938). The absence of a sucker in *A. acroporae* is surprising, given the difficulty of removing specimens from the coral. Perhaps, their oval, stout, concave body shape creates a more efficient suction to the rugose coral surface than would the sucker organ that is typical of other cotylean polyclads.

The validity of genera within Prosthiostomidae has been a matter of contention among polyclad taxonomists. Hyman (1959), in her study of prosthiostomids, doubted the validity of *Amakusaplana* as a genus and Faubel (1984) synonymized *Amakusaplana* with *Prosthiostomum*, citing an absence of sufficient morphological grounds for maintaining these as distinct genera. However, Poulter (1975) and Prudhoe (1985) support Kato's (1938) original erection of the genus *Amakusaplana*, based on body shape, eye arrangement and, most importantly, the absence of a ventral sucker organ. We therefore recognize *Amakusaplana* as valid, based on these characters.

The comparative analysis of polyclad 28S rDNA sequences independently verified *Amakusaplana acroporae* as a sucker-less cotylean. The presence or absence of a sucker on the ventral surface has been used historically to distinguish between polyclad suborders (Lang 1884). The acotyleans generally lack a sucker, whereas the cotyleans possess a sucker at varying positions along the ventral midline posterior to the female gonopore, though with the following *caveats*: in addition to *Amakusaplana ohshimai*,

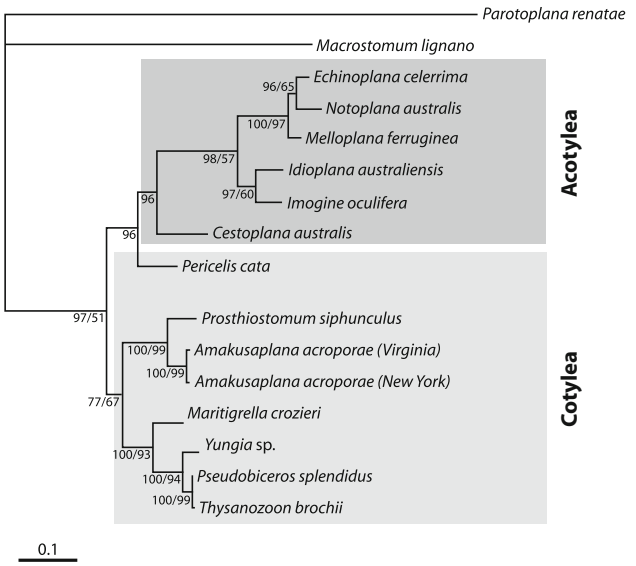

Fig. 7 Phylogenetic tree resulting from the Bayesian analysis of 28S rDNA sequence data. Clade support indicated by Bayesian posterior probabilities/Bootstrap values from maximum likelihood analysis (where available). Suborders Cotylea and Acotylea (sensu Lang 1884) are indicated on the right. *Amakusaplana acroporae* resolves as sister to *Prosthiostomum siphunculus* within the Cotylea

six other cotylean species appear to lack ventral suckers (*Diplopharyngeata filiformis*, Plehn 1896; *Simpliciplana marginata*, Kaburaki 1923; *Diposthus corallicola,* Woodworth 1898; *D. popae*, Hyman 1959; *Nymphozoon bayeri,* Hyman 1959; *Chromyella saga,* Correa 1958), though some of these descriptions were based on damaged specimens. Interestingly, two acotylean species – *Leptoplana tremellaris,* Muller 1774 and *Itannia ornata* Marcus 1947 – show genital suckers, a likely convergence on the cotylean condition. Finally, a sucker in the form of an adhesive disc is found in the boniniid (cotyleans) (Bock 1923) and some cestoplanid (acotyleans) (Lang 1884) polyclads.

As it stands, the phylogenetic distribution of a ventral midline sucker on our 28S rDNA tree (Fig. 7) suggests that *Amakusaplana* species have secondarily lost this structure. This organ of attachment is present in the sister taxon, *Prosthiostomum*, and the sister clade (*Maritigrella crozieri, Yungia* sp., *Pseudobiceros splendidus and Thysanozoon brocchii*). Indeed, our cursory analysis – which resolves *Pericelis cata* as sister to the Acotylea (albeit with weak bootstrap support) – would suggest that the presence of a sucker might, in fact, represent the plesiomorphic condition for the Polycladida, with the sucker having been lost along the lineage leading to Acotylea. However, given the considerable variation in polyclad sucker morphology (see above), a careful revision of the structure, histology, development and phylogenetic distribution of polyclad sucker organs is needed before a robust sequence of character evolution may be proposed. Furthermore, while our single gene tree supports the generic level relationships of the Cotylea as proposed by Rawlinson and Litvaitis (2008), much greater taxon sampling – and data from multiple genetic loci – is needed to resolve the deeper level interrelationships of the Polycladida and to rigorously test the monophyly of the cotyleans and acotyleans, as defined by Lang (1884).

The short, cleft, tubular pharynx of *Amakusaplana acroporae* resembles that found in the coral ectoparasite *Prosthiostomum (Prosthiostomum) montiporae* (Poulter 1975), and this may be distinct from the morphology of tubular pharynx found in other prosthiostomids. The pharyngeal morphology *of Amakusaplana ohshimai* was not discussed by Kato (1938), so the possible ecological significance and phylogenetic distribution of a cleft pharynx within prosthiostomids remain unclear. Poulter (1975) proposed that the cleft pharynx is an adaptation to corallivory and that it may be employed as a typical tubular pharynx or, once protruded, may be opened along the deep cleft and spread over a broad or uneven area for more efficient feeding. While the presence of a cleft pharynx in the corallivorous *Amakusaplana acroporae* is consistent with this, a survey of pharyngeal structure in additional (non-corallivorous) prosthiostomid taxa is needed to further test this adaptive hypothesis. The Prosthiostomidae is a diverse and understudied polyclad group, including members that exhibit diverse feeding strategies ranging from general predation to coral ectoparasitism (Jokiel and Townsley 1974). This group therefore offers an exceptional opportunity to test hypotheses of morphological and life history (see below) adaptation to prey specificity.

Life history strategy

Cleared whole mounts and histological sections of adult worms reveal considerable egg production, though how this fecundity compares to other polyclad species is unknown. In closed aquarium systems, the natural predators of *Amakusaplana acroporae* adults, juveniles and eggs may be absent allowing numbers to increase to levels where coral colony mortality is recorded. There is anecdotal evidence that some fish species (*Halichoeres chrysus, H. iridis, Macropharyngodon ornatus, Labroides dimidiatus, Synchiropus ocellatus* and *S. splendidus*) prey on the adult worms in aquaria (Jason Jenkins, pers comm.). The adult's camouflage against the coral tissue and the hatchling's ability to swim into the coral skeleton may be strategies to avoid predation.

Amakusaplana acroporae exhibits an intermediate mode of development, in which embryos exhibit anatomical characters typical of a pelagic life history stage within the egg and undergo 'metamorphosis' prior to hatching. Intermediate development in the form of an intracapsular

'larva' has been described in one other polyclad to date, the acotylean *Planocera reticulata* Kato 1940, making *A. acroporae* the first example of intermediate development in a cotylean polyclad. Also common to *Planocera reticulata* and *Amakusaplana acroporae* is the presence of multiple embryos per egg capsule, though this feature is also found in many other members of the Prosthiostomidae (*Prosthiostomum siphunculus*, Lang 1884; *Prosthiostomum (P) montiporae*, Jokiel and Townsley 1974; *Enchiridium periommatum*, pers. obs.), as well as in certain pericelid and boninid polyclads (pers. obs.). With the exception of *A. acroporae*, however, all of the cotylean taxa exhibiting multiple embryos per egg capsule exhibit indirect development. In the light of the observed 100% intracapsular metamorphosis in *A. acroporae*, and the occurrence of indirect development in other (i.e. non-*Amakusplana*) prosthiostomids, it is most parsimonious to propose that the pelagic life history phase of *A. acroporae* has been lost due to a heterochronic shift in either the timing of metamorphosis (i.e. metamorphosis occurs earlier, prior to hatching) or the timing of hatching (i.e. hatching has been delayed until after metamorphosis). In either case, the consequence would be reduced time spent in the water column and increased retention of hatchlings within the natal habitat. In *A. acroporae*, limited dispersal potential may have evolved in concert with prey specificity (i.e. corallivory). However, a thorough sampling of prosthiostomid life history strategies and feeding ecology will be needed to test this phylogenetic hypothesis.

The retention of lobes and ciliary tufts during embryonic development begs the question: are these features non-functional evolutionary vestiges, or have these characters (which would normally facilitate prolonged dispersal) been retained as a 'bet-hedging' adaptation to spatially and temporally patchy resources? In numerous sacoglossan and nudibranch opisthobranchs, strong ecological ties to a patchy and unpredictable resource (a specific host algae) have likely driven the evolution of dispersal dimorphisms (Krug 2009). Furthermore, such bet-hedging strategies may also exist in *Planocera reticulata*, the only other reported instance of polyclad intermediate development. While Kato (1938) reported exclusive intracapsular metamorphosis in *P. reticulata*, Teshirogi et al. (1981) reported *P. reticulata* hatching as both a pelagic lobed larva and directly as a juvenile. It will be important to determine whether post-hatching metamorphosis occurs in *A. acroporae*, and if so, to assess the frequencies of pre- and post-hatching metamorphosis in aquaria and in the field. As *A.acroporae* appears to have strong ecological ties with certain host acroporids that, in the wild, inhabit shallow subtidal environments with rapidly fluctuating conditions, it may be advantageous to retain a spectrum of dispersal strategies that will vary in fitness depending on whether selection favours local retention or dispersal away from the natal habitat.

Our study of the AEFW, *Amakusaplana acroporae*, has highlighted morphological (absence of a sucker, cleft pharynx) and life history (intracapsular larva) conditions that might represent adaptations to prey specificity on acroporid corals. These conditions, along with cryptic camouflage and the ability to reproduce in large numbers in aquaria, pose difficulties for the maintenance of healthy acroporid colonies in captivity following an *A. acroporae* infestation. Currently recommended treatments to reduce *A. acroporae* numbers include spraying freshwater onto the corals to loosen adults, the introduction of wrasse (e.g. *Halichoeres* spp.) to prey on loosened adults in the water column and the removal of egg capsules, where possible (Nosratpour 2008). It is our hope that further observations on the biology and ecological interactions of *A. acroporae* in its natural environment may shed light on alternative – and more effective – biological controls.

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