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A new solar powered species of the genus *Phyllodesmium* Ehrenberg, 1831 (Mollusca: Nudibranchia: Aeolidoidea) from Indonesia with analysis of its photosynthetic activity and notes on biology

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Abstract

A new *Phyllodesmium* species, *P. jakobsenae* nov. sp., is described from North Sulawesi, Indonesia. The new species is associated with the octocoral *Xenia* sp. Its external morphology is similar to other *Phyllodesmium* species inhabiting *Xenia* colonies, especially to *P. hyalinum* Ehrenberg, 1831, but cerata morphology and color, tooth and jaw morphology as well as the digestive glandular branching system within the cerata clearly distinguishes this new species. Behavioural notes are given and spawning is described. Active photosynthesis due to zooxanthellae (Dinophyceae of the genus *Symbiodinium*) in the digestive gland was measured *in situ* with a Diving-PAM (Pulse Amplitude Modulated Fluorometer). Experiments with *P. jakobsenae* indicate a symbiotic relationship with zooxanthellae at least for some days. These results are discussed in comparison to another *Xenia* inhabiting species, *Phyllodesmium crypticum* Rudman, 1981. Histological investigation of the digestive diverticula within the cerata of the new species also indicate a high effectiveness of the symbiosis.

Key words: *Phyllodesmium*, zooxanthellae, *Symbiodinium*, *Xenia*, solar powered, mutualistic symbiosis, Diving-PAM, photosynthesis, Sulawesi

Introduction

Up to now 15 species of the genus *Phyllodesmium* Ehrenberg, 1831 (Facelinidae, Aeolidoidea) have been described, (see Rudman 1981, 1991, Baba 1949, 1991, Avila et al. 1998, Ortiz & Gosliner 2003). For additional 11 undescribed species from the Indopacific, information is available in the Internet (Rudman 2004, Seaslugforum: www.seaslugforum.net). The first author found another undescribed species during a stay on Sulawesi (Indopacific) in summer 2003. Compared to other genera of the Facelinidae, *Phyllodes-*

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mium with at least 27 species is relatively diverse. The species of *Phyllodesmium* are mainly distinguished by different external color patterns, the shape of the cerata, digestive gland ramification, differences in radular morphology and anal position (for details see genus diagnosis below). Additionally, most but not all species house zooxanthellae inside their digestive glandular cells. All feed on octocorals, but some seem to be specialised in feeding on certain coral genera (Rudman 1981, 1991; Wägele & Johnsen 2001).

The first aim of the present publication is to describe the new species of *Phyllodesmium* from Bunaken Island (Sulawesi, Indonesia) and to discuss some aspects of its ecology.

The second aim of this study is to clarify the mutualistic relationship with zooxanthellae and to test the hypotheses of Rudman (1981, 1991) for the new *Xenia* feeding species. Rudman (1981, 1991) already discussed the role of a possible symbiotic relationship between some members of *Phyllodesmium* and zooxanthellae of the genus *Symbiodinium*. According to this author, a mutualistic symbiosis has the following advantages: at least color camouflage (crypsis) and the availability of additional nutrients (photosynthetic products). Whereas Rudman (1981, 1991) based his assumptions on histological results, Wägele & Johnsen (2001) were able to measure the photosynthetic activity of the zooxanthellae within *Phyllodesmium briareum* Ehrenberg, 1831. Rudman (1991) stated that there is a correlation between the grade of branching of the digestive gland and the efficiency of the symbiosis. Species with a higher ramification of the digestive gland (e.g. “secondary” branching) are assumed to be derived and adapted to a symbiotic relationship. He assumed that *Xenia* feeding species show a less developed stage of symbiosis, according to their less developed digestive glandular structures.

Therefore the new species was kept under starving conditions in aquaria and measurements of possible photosynthesis have been taken with the help of a Diving-PAM (Pulse Amplitude Modulated Fluorometer). Additionally the structure of the digestive gland was investigated by histological methods.

Material and methods

Specimens were collected by hand at Bunaken Island (North Sulawesi) in July 2003 while snorkeling along reef flats. All specimens were kept in aquaria for some days (see Tab. 1) in order to perform starving experiments and to investigate a possible symbiotic relationship with zooxanthellae. Measurements of photosynthetic activity were taken with the help of a Pulse Amplitude Modulated Fluorometer (Diving-PAM, WALZ, Germany). The Diving-PAM measures the fluorescence emitted by Photosystem II in chlorophyll a of chloroplasts and allows to distinguish living zooxanthellae from digested ones in an ecophysiological way. The method was already applied on other Opisthobranchia (Wägele & Johnsen 2001). For details see Wägele & Johnsen (2001). Animals were directly preserved in formalin 37%/ seawater (1:4) and transferred to ethanol for dissection and histology, or preserved in 70 % ethanol. Dissections of the general anatomy were made of three specimens (see Table 1). One animal (No. 4) and additional cerata of specimen No. 1 were embedded in hydroxyethylmethacrylate for histological serial sections (2.5 µm, stained with toluidine blue). Pictures were taken with a digital camera (Olympus DP 50) on an Olympus microscope. The hard structures of the digestive system (jaws and radula) were prepared for investigations with a SEM (Zeiss DSM 950).

TABLE 1: Specimens collected during July 2003 in Northern Sulawesi, with some individual dates. Availability of specimens indicated in last column. Numbers refer to institutions:

Specimen	Lenght (living animal)	Length (pres. animal)	Capture/ Cultivation	Comments	Depository of specimen
# 1	30 mm	13 mm	07/12/ - 07/20/2003	Dissected; Histological sections of cerata	Bochum [2]
# 2	-	10 mm	07/12/ - 07/20/2003	-	Bochum [2]
# 3	-	10 mm	07/12/ - 07/20/2003	Dissected completely; SEM: Radula and jaws	Bochum [2]
# 4	-	5 mm	07/12/ - 07/21/2003	Histological preparation of whole animal	Bochum [2]
# 5	29 mm	11 mm	07/12/ - 07/17/2003	Dissected completely; SEM: Radula and jaws	Bochum [2]
# 6	-	7 mm	07/12/ - 07/17/2003	-	Bochum [2]
# 7	-	10 mm	Fixed on 07/12/2003	-	Holotype[1]: ZSM Moll 20040189
# 8	25 mm	8 mm	07/20/-08/01/2003	-	Paratype[1]: ZSM Moll 20040190

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Phyllodesmium **Ehrenberg, 1831** (type species: *P. hyalinum*)

Diagnosis of the genus according to Rudman 1981:

Alcyonarian-eating aeolids with readily autotomizing cerata. Cerata slightly or extremely flattened, lacking functional cnidosacs. Oral glands absent, a pair of discrete tubular salivary glands present; ceratal arrangement variable with precardiac arch on each side either single- or double-rowed. Post-cardiac cerata arranged in single- or double-rowed arches, simple rows, or a mixture of single-rowed arches and simple rows. Cleioproctic, anus lying in first post-cardiac arch or behind first post-cardiac row or in one case above post-cardiac arch. Genital opening below anterior limb of right pre-cardiac arch. Radula formula 0.1.0. Teeth usually with long pointed central cusp with a lateral flange down each side bearing many denticles or serrations. Occasionally the serrations may be absent, or strongly developed into long thin tubular denticles. Masticatory process of jaws slightly roughened or developed into strong pointed serrations. Reproductive system with single allosperm receptacle and prostate forming gland mass at base of muscular penis. At present only known from the Indo-West Pacific.

Phyllodesmium jakobsenae **nov. sp.**

Figures 1–5

Repository

ZSM ñ Zoologische Staatssammlung München, Germany (Zoological State Collection Munich):

Holotype: ZSM Moll 20040189, Paratype: ZSM Moll 20040190

Localities

Pulau Bunaken (Bunaken Island, Bunaken Islands National Park), North Sulawesi (Indonesia). Seven specimens (including the holotype) found in the lagoon in front of "Papa Boa Bungalows" on the southern side of Bunaken Island (07/12/2003; Western end of Liang Beach, GPS: 01°37'3.1"N, 124°45'51.1"E; 0.3–0.5 m depth). One specimen found in front of the South West cape of Bunaken Island (07/20/2003; 0.5 m depth).

Ethymology

This species is dedicated to Mrs. Wera Jakobsen, a passionate diver, who supported alpha-taxonomy of marine slugs by a donation to BIOPAT (Patrons for Biodiversity).

Description

Color and external morphology of living animal: Body of living animals, including oral tentacles, rhinophores and foot translucent white (Fig. 1A–E). Gonads shining through translucent epidermis in pale yellow (Fig. 1A), buccal bulb in light orange.

Animals elongate, up to approximately 30 mm in length (without cerata), with a few short and several very long cerata (longer than rhinophores and oral tentacles), covering whole notum (Fig. 1C–E). Smooth oral tentacles slightly longer than rhinophores, usually directed laterally (Fig. 1C). Rhinophores similar in shape and surface texture to oral tentacles, standing close together. Both rhinophores and oral tentacles tapering. Anterior foot angular, slightly extended and with some white pigmentation mainly along anterior edge. Posterior end of foot tapering.

Cerata (25–35) arranged in clusters of up to 7 on each pad. Compared to more dorsal ones, lateral cerata in most specimens very small and short. Posterior cerata appearing longer than more anterior ones (Fig. 1C). Upper third of elongate, dorso-ventrally flattened ceras spatulate or rather arrowhead-shaped. In middle of broadened part white longitudinal stripe present, extending from base of ceras to tip and running parallel to main digestive glandular duct. Lateral of this central whitish stripe one broad brownish longitudinal stripe on each side (Fig. 1C–E), composed of very small singular brownish dots, probably representing clusters of zooxanthellae within digestive gland. In some specimens brownish stripes riddled with nodulose white markings (dots or longitudinal lines). Edge of ceras appearing whitish and slightly nodulose, tips blunt and rounded. Cerata usually partly curled distally. Basal whitish part of ceras more or less circular in cross-section, smooth with distinct raised ridge on ventral midline of proximal third of each ceras. Ridge continuing into white line of upper part of ceras. Basal end of ceras appearing clubbed, tapering slightly more distally before broadening abruptly into arrowhead-shaped apical part. Thin longitudinal, creamy colored lines on this part of ceras.

Description of preserved animals

External morphology: Due to starving condition, many animals have shrunken considerably. Nearly all cerata repelled, only small ones still attached to body. No distinct notal rim present. Foot anteriorly without propodial tentacles, posterior part pointed. Oral tentacles wrinkled, slightly longer than rhinophores, with tiny groove on dorsal side. Rhinophores heavily wrinkled, giving the appearance of being lamellate, and standing close to each other (Fig. 2A). Larger cerata of preserved animals nearly as long as body. Insertion of cerata difficult to see in preserved specimens, probably arranged in arches, but lying on distinct pads. Four pads on each side, lying opposite to each other. First cerata lateral of rhinophores. Anal papilla dorsally on right side behind second pad (Fig. 2A). Genital opening below first cerata pad. Whole epidermis composed of specialized vacuolated cells (Figs. 4C–E).

Central nervous system: Central nervous system located behind pharynx (Fig. 2B). Cerebral and pleural ganglia completely fused (Fig. 2F). Buccal ganglia lying beneath oesophagus at junction with pharynx. Large rhinophoral ganglion at base of each rhinophore, connected to cerebral ganglion by short connective. Eyes situated directly at cerebral ganglia (Fig. 2F), of usual nudibranch arrangement with pigment and lense (Fig. 4F). Statocysts lying between cerebropleural and pedal ganglia. Only one statolith present (Fig. 4F)

Digestive system: Oral tube short, leading into bulbous pharynx (Fig. 2B). Huge oral glands present (Fig. 4G), forming many lobes around pharynx. Glandular tissue composed of cells with blue to violet staining contents, indicating acid and neutral mucopolysaccharides. Paired outleading ducts from each side uniting beneath pharynx and opening into very short oral tube. Labial cuticle without any armature. Two jaw plates oval shaped (Figs. 2C, 3A). Along cutting edge of jaw plates up to 9 somewhat hollowed and clog-shaped denticles present, getting progressively longer in size (Figs. 3A, B). Longest denticles about 300µm in length. Radular formula of two specimens 39 x 0.1.0 (No.3) and 40 x

0.1.0. (No.5). Each rhachidian tooth with large (50µm long) median pointed cusp (Figs. 3C–G), claw-shaped in lateral view (Figs. 3C, D). Margin of rhachidians with about 38 to 43 relatively short (5 to 10µm long) denticles (Figs. 3 E–G). In anterior part of radula of specimen No. 5 denticles flattened, showing attrition (Figs. 3C, D). Salivary glands could not be found either by dissection or in histological slides. Oesophagus short, entering stomach on ventral side (Fig. 2B). Interior of oesophagus completely covered by specialized vacuolated cells. Stomach and posterior part of digestive gland not separated, characterized by many longitudinal folds. Two anterior digestive glandular branches starting above entrance of oesophagus and leading to first cerata pads on both sides. Right duct orange to brown and therefore differing in color compared to all other main digestive glandular ducts, which are translucent to whitish. One main duct leading to posterior part of body with opposite lying ducts leading into posterior cerata pads. Intestine starting next to oesophagus and anterior digestive glandular branches, leading on the right side to anus (Fig. 2B). No typhlosolis in intestine observed in dissected animals, but small one present in histological slides.

Digestive gland in cerata: One central narrow digestive gland duct extending through each ceras. Especially in apical, broadened part, central duct ramifying in primary and secondary branches, the latter radiating towards ceratal wall (Figs. 2E, 4B). Each secondary branch terminating in sac-like structure. On side of flattened part of ceras not exposed to light, sacs concentrated only at margin of each ceras (lower area in Fig. 2E, Fig. 4B). A stripe free of these sacs parallel to central duct, with white pigmentation. Light orientated side of each ceras (Fig. 2E upper part, Fig. 4A) densely packed with sacs directly beneath ceratal wall. Central stripe with secondary branchings not ending in sacs but in thickened terminations also present, but much smaller. Zooxanthellae present in digestive glandular cells of these sacs, in lumen of sacs, and in epithelium of branches (Figs. 4C, D). Cnidosac of these large cerata with cells containing one large vacuole. No contents observed in these cells. Distinct muscular layer around cnidosac present.

Small cerata, which did not autotomize, showing different morphology and histology: central digestive glandular branch not ramifying, occupied by many glandular cells. Cnidosac cells filled with tiny nematocyst like structures, arranged around central lumen. Beneath ceras epithelium small glandular cells (“cellules spéciales” see Edmunds 1966: 34) present (Fig. 4E), lacking in the larger cerata. Epithelial glandular mucous cells present in smaller cerata (Fig.4E).

Excretory system: Syrinx large, inside highly folded, opening ventrally into pericard on anterior right side (Fig. 2A).

Circulatory system: Ventricle medio-dorsal between first and second cerata pad. Atrium lying behind ventricle, both arranged in longitudinal direction (Fig. 2A).

Reproductive system: Gonad follicles not arranged into lobes but forming rather uniform layer lying beneath digestive gland. Gonad reaching into anterior third of ventral visceral cavity. Male and female follicles separate. Distal genital system occupying whole anterior part of visceral cavity. Gonoduct, with sausage-shaped ampulla, dividing into vas deferens and oviduct next to atrium, separation lying inside of nidamental glands (Fig. 2D). Vas deferens soon widening into sausage-shaped prostate, forming one large coil, ending in one small papilliform penis. Receptaculum seminis slightly enlongate, entering atrium next to oviduct opening.

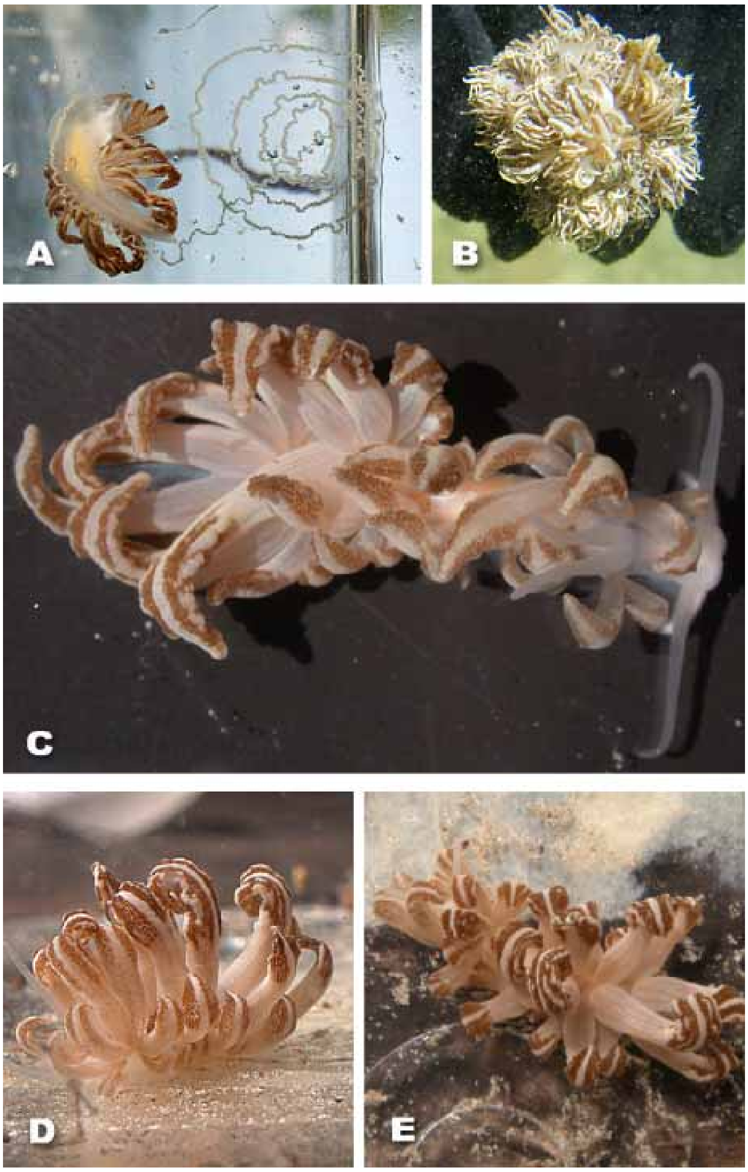

FIGURE 1: *Phyllodesmium jakobsenae*, living animals from North Sulawesi: A: Specimen laying eggs in an aquarium. B: Two specimens sitting in their food coral *Xenia*: on the right side a specimen with more brownish cerata and to the left a bigger specimen with more whitish cerata. Polyps of *Xenia* surround both individuals. C: Bigger specimen from B; please note the smaller cerata in the anterior part of body and the oral tentacles stretched to the lateral sides. D: Animal sitting inactive and mimicking *Xenia* polyps. E: Specimen starting to crawl.

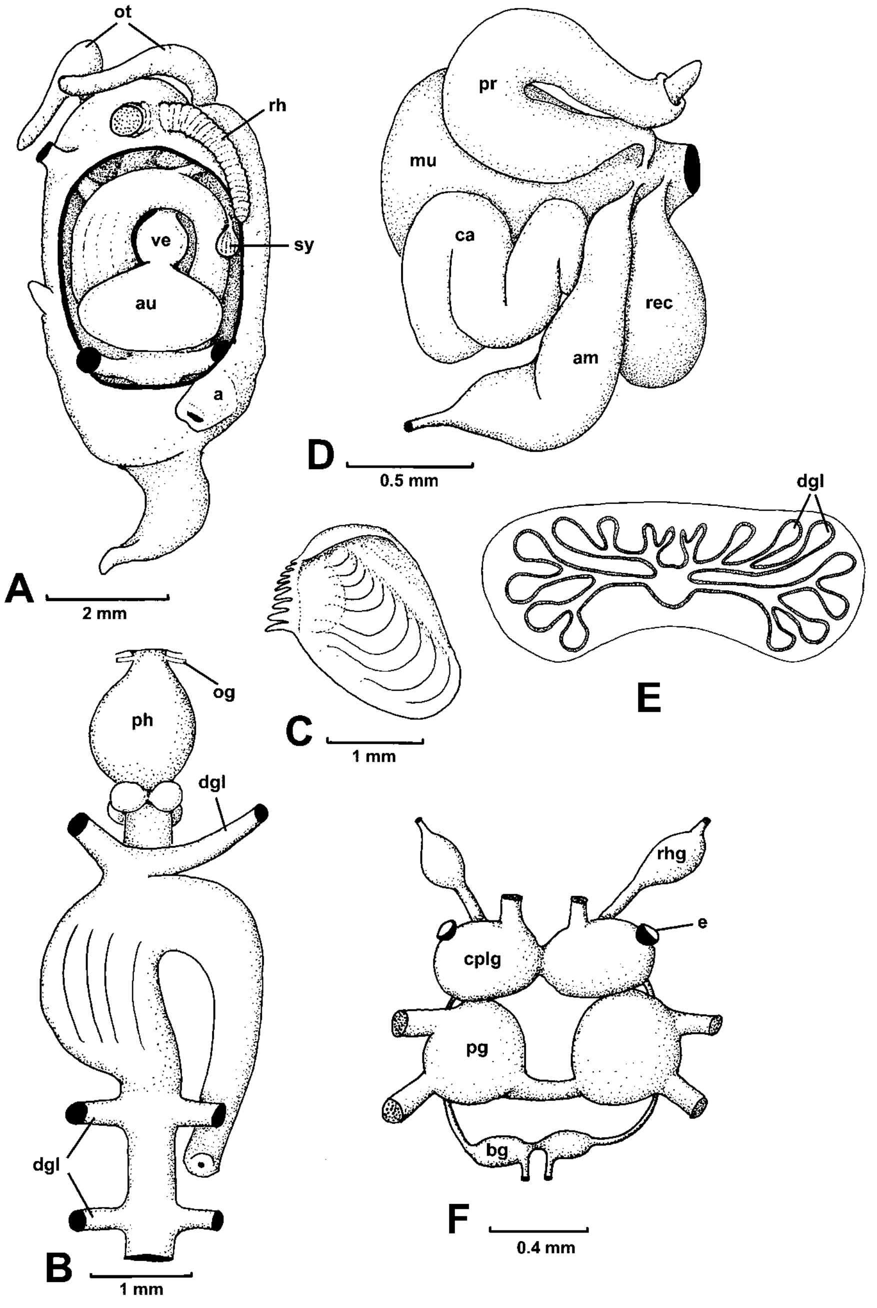

FIGURE 2: *Phyllodesmium jakobsenae*, morphology: A: General outline of organs *in situ*. B: Digestive tract (oral glands omitted). C: Right jaw seen from the interior side. D: Distal genital system. E: Schematic cross section of apical part of ceras. The upper side represents the part which is exposed to light, when resting on the food coral. F: Central nervous system. Abbreviations: a anus, am ampulla, au auricle, bg buccal ganglion, ca capsule gland, cplg cerebropleural ganglion, dgl digestive gland, e eye, i intestine, mu mucous gland, og oral glands, ot oral tentacles, pg pedal ganglion, ph pharynx, pr prostate, rec receptaculum seminis, rh rhinophore, rhg rhinophoral ganglion, sy syrinx, ve ventricle.

Biological notes

All specimens were found nestled into coral colonies of an unknown species of *Xenia* (Ehrenberg, 1831; Xeniidae, Alcyonaria, Octocorallia). The bodies of the slugs were not visible, only the cerata were slightly surpassing the coral's tentacles. The animals are very well camouflaged, the cerata mimicking parts of the tentacles of *Xenia* in color and shape. One to three specimens were found in each *Xenia* colony. Figure 1B shows one *Xenia* colony with two slugs sitting in the middle. Part of the colony is preserved in formalin/ seawater and kept together with the holotype.

The locality in front of "Papa Boa Bungalows" is a shallow lagoon fringed by mangroves. All specimens were found in the intertidal zone between 0.3 and 0.5 m depth. The substrate was a mixture of sand, mud, coral rubble and some living hard corals. On the coral rubble there were single colonies of *Xenia*. Most of the sandy patches around the *Xenia* colonies were covered with seagrass. Water temperature at locality was 31°C on average (July 2003).

The second locality close the South West cape of Bunaken Island is a coral reef with sandy patches in between. On these sandy patches the slugs were also sitting in *Xenia* attached to coral rubble.

With the exception of the holotype, all specimens were immediately put into aquaria in order to perform long-term starvation experiments. All specimens shrunk considerably after some days and autotomized most of their cerata. Especially the arrowhead-shaped and broadened apical parts of the remaining cerata shrunk after some days. Observations in the aquarium showed an interesting diurnal behaviour: During daytime, the specimens mainly remained inactive. The long narrowed base of the cerata was often extremely contracted and shortened in most of the specimens. Additionally the apical, arrowhead-shaped part of the cerata was extremely broadened. During nighttime the slugs became more active and moved through the aquarium. The bases of the cerata were prolonged again and the upper part of the cerata was less broad than before. The cerata easily autotomized when the animals were disturbed. Detached cerata exuded a sticky secretion and moved for some minutes. This secretion may have come from the few mucous cells found in the epidermis and probably not from the "cellules spéciales" (Edmunds 1966: 34) since they have not been observed in the large cerata.

Some specimens laid spawn masses after one or two days of capture. The spawn mass is a whitish ribbon-like cord, with egg capsules arranged in a line. It is attached to the substrate in form of a spiral (3 to 4 cm in diameter) (Fig. 1A). After 5 to 6 days kept in the aquarium (average temperature 29–31°C) the free swimming veliger larvae hatched.

Measurements of photosynthetic activity (PAM)

Figure 5A shows the yield values of *Phyllodesmium jakobsenae, P. crypticum* Rudman, 1981 and the octocoral *Xenia* sp. plotted versus the irradiance. Both *Phyllodesmium* species were feeding on probably the same species of *Xenia* but in different localities: *P.*

jakobsenae was found close to Bunaken Island, whereas *P. crypticum* was found in Lembeh Strait, North Sulawesi. The yield values of both *Phyllodesmium* species decrease exponentially with increase of irradiance, and the values of *P. crypticum* are nearly identical to the ones of *P. jakobsenae*. In lower irradiances the values of *Xenia* sp. are only slightly higher than those of the slugs, but around 500 µmol and higher, values for the soft coral are significantly higher. In Figure 5B, the yield values of one specimen of *P. jakobsenae* (No.8) are plotted versus the number of cultivation days under starving conditions in the aquarium. The yield values stay on a high level between 0.5 and 0.7 for the whole ten days of the experiments. In Figure 5C, the ground fluorescence (F_0) values of one specimen of *P. jakobsenae* (No.1) are plotted versus the number of cultivation days under starving conditions in the aquarium. The F_0 values decrease quickly from an average of 350 in the beginning to approximately 100 after 8 days.

Discussion

Taxonomic discussion

According to the features outlined by Rudman (1981) for the genus *Phyllodesmium*, the new species can be assigned to that genus: Reproductive system similar to all other described species of *Phyllodesmium*; shape of the radular tooth and jaws very similar; replacement of the cnidosac by a terminal sac without cnides at least in the large cerata; easily autotomising cerata, that wriggle and exude a sticky secretion when autotomised. Additionally the new species also feeds on alcyonarian octocorals (*Xenia* sp.). Rudman (1981) stated the absence of oral glands as a generic feature. This has to be changed in the light of this study. *Phyllodesmium jakobsenae* definitely has large oral glands (Fig. 4G), but no salivary glands were found. The previous descriptions (Avila et al. 1998, Baba 1991, Ortiz & Gosliner 2003, Rudman 1981, 1991) concerning presence of salivary glands and absence of oral glands have to be reinvestigated.

Externally, *Phyllodesmium jakobsenae* is similar to the other three *Xenia*-feeding species of *Phyllodesmium*: *P. crypticum*, *P. hyalinum* and *P. pecten* Rudman, 1981. They all mimic the tentacles of the corals and are very cryptic. All *Xenia* feeders, including the new species, show very strong denticles on the masticatory border of the jaws. This could be directly correlated to feeding on *Xenia*, as has been discussed by Rudman (1981) for *P. crypticum*, *P. hyalinum* and *P. pecten*. In all four species living on *Xenia*, the cerata, the jaws and the radula look rather similar. These features of the buccal armature and the fact that they are restricted to feed on *Xenia* corals clearly separate all *Xenia* feeders from the other species of the genus.

Despite the similarities, *P. jakobsenae* can be clearly distinguished from other *Xenia* feeders by several characters: There is a coarse serration of the cutting edge of the jaw plate in *P. crypticum*, that is missing in *P. jakobsenae*. Additionally the teeth of the cutting edge of the jaw plates elongate progressively in size in *P. jakobsenae*, whereas the denticles of *P. crypticum* are irregular in length. In *P. crypticum* the cerata are quadrangular in cross section and very nodulose, especially along the edges. In *P. pecten* the cerata are semicircular in cross section. The shape of the cerata of *P. jakobsenae* will be discussed below. The anal papilla of *P. crypticum* and *P. pecten* lies inside the second ceratal arch. In *P. jakobsenae* the anal papilla lies more dorsally, outside the second ceratal patch. In *P. crypticum* the anterior corners of the foot are developed into tentacular processes, in *P. jakobsenae* they are not. The length of the rhinophores in *P. pecten* and *P. crypticum* is only two thirds of the length of the oral tentacles, whereas in *P. jakobsenae* the rhinophores are only slightly shorter than the oral tentacles. *P. crypticum* has only been found feeding on top of *Xenia* colonies, *P. jakobsenae* was always found burrowed deeply inside *Xenia*.

Phyllodesmium hyalinum shares several similarities with the new species. Probably correlated to that habit, both species have a more dorsally situated anal papilla than the other *Xenia* feeders and burrow into the fleshy trunk of *Xenia*. Also the ducts of the digestive gland inside the cerata branches on a secondary level in both species, whereas in *P. crypticum* and *P. pecten* it is only on a primary level. Differences that distinguish *P. jakobsenae* clearly from *P. hyalinum* are details of the shape of the cerata. In *P. jakobsenae,* the ceras is divided into a cylindrical basal part and a dorso-ventrally flattened apical part with a distinct color pattern of white and brown stripes. In *P. hyalinum* there is no distinct color pattern in the apical part of the ceras, it is more or less uniformly brownish colored and the ceras is also slightly flattened at the base. Furthermore the nodules on the cerata of *P. hyalinum* are spread over the whole ceras, whereas they are concentrated on the edges of the ceras in *P. jakobsenae.* In both species the digestive gland inside the cerata is secondarily branched, but in *P. hyalinum* the fine branches orientate star-like to all sides of the ceras. In *P. jakobsenae* the branching of the digestive gland shows unique features for the *Xenia*-feeding *Phyllodesmium* species: The fine ducts terminate in sac-like structures and these are mainly concentrated on the flattened side of the ceras orientated towards the light. Both species also differ in the jaws and radula. In *P. hyalinum* the denticles on the masticatory border of the jaws are more straight lined, whereas those of *P. jakobsenae* show depressions and are clog-shaped. The jaw plates of *P. jakobsenae* are not characteristically bilobed in outline like in *P. hyalinum*. The main cusp of the rhachidian teeth in *P. hyalinum* is also more straight lined, whereas the ones in *P. jakobsenae* are hook shaped and look like a claw. Certain patches of white pigmentation just behind the rhinophores and over the pericardium are often present in *P. hyalinum*, but absent in *P. jakobsenae*.

Beside the *Xenia* feeding species of *Phyllodesmium,* the other species of the genus have morphological, anatomical and ecological differences that clearly distinguish them from *P. jakobsenae* (see Tab.2). All described species of *Phyllodesmium* house zooxanthellae inside of their digestive glandular cells, except for five species. These five species can already be separated by their digestive glandular system, which shows no or just a slight branching within the cerata.

TABLE 2: Comparison of relevant external and anatomical features of valid non-*Xenia* feeding *Phyllodesmium* species with *Phyllodesmium jakobsenae*

	Characters							
Phyllodesmium species	1	2	3	4	5	6	7	8
P. jakobsenae **nov. sp.**	+	+	+	+	+	+	-	+
P. briareum (Bergh, 1896)	+	-	+	-	+	+	-	-
P. colemani Rudman, 1991	+	-	+	+	+	+	+	-
P. guamensis Avila et al., 1998	+	-	+	+	+	-	-	-
P. horridum (Macnae, 1954)	-	-	+	-	-	-	+	-
P. iriomotense Baba, 1991	-	-	+	-	-	-	-	-
P. kabiranum Baba, 1991	+	-	+	+	+	-	+	-
P. longicirrum (Bergh, 1905)	+	-	-	+	+	+	-	-
P. macphersonae (Burn, 1962)	+	-	+	-	+	+	-	-
P. magnum Rudman, 1991	+	-	-	+	+	+	-	-
P. opalescens Rudman, 1991	-	-	+	-	-	-	+	-
P. parangatum Ortiz & Gosliner, 2003	?	-	+	+	+	-	-	-
P. poindimiei (Risbec, 1928)	-	-	+	-	-	-	-	-
P. serratum (Baba, 1949)	-	-	+	-	-	-	-	-

Numbers refer to different characters: 1: Zooxanthellae incorporated; 2: Presence of strong serrations/ denticles on the jaws; 3: Presence of well-developed row of denticles on the teeth of the radula; 4: Flattened cerata; 5: Branching of digestive gland inside cerata secondary; 6: Presence of sacs at the ends of the fine branches of the digestive gland for storing of zooxanthellae; 7: Presence of white patches/ pigmentation on body; 8: Anal papilla lying dorsally.

A well-developed row of denticles on the teeth of the radula are replaced by lamellae in *P. longicirrum* (Bergh, 1905). In *P. guamensis* Avila et al., 1998 the denticles are arranged in double rows instead of a single row.

The cerata of *P. jakobsenae* are cylindrical in the basal part and dorso-ventrally flattened in the distal part. In combination with the coloration of these parts of the ceras (basal part whitish, distal part with brown and white stripes) this is unique in *Phyllodesmium*.

P. jakobsenae is the only species with zooxanthellae (besides the other *Xenia* feeding species) that does not show any branching of the digestive gland inside the body, only inside the cerata. Compared to the other species (besides *P. hyalinum*) the anal papilla in *P. jakobsenae* lies more dorsally (outside the insertion of the cerata). About the exact arrangement and insertion of the cerata of *P. jakobsenae* we cannot make a clear statement, because it was not visible in the living animals, neither in the preserved ones. The cerata are grouped in clusters, but we can only assume that they are arranged in arches. Noteworthy is the presence of two different kinds of cerata. The smaller ones do not seem to be adapted to house zooxanthellae yet, since they show no branching and no zooxanthellae were detected. It is also very astonishing that small cnidocysts are found in the cnidosac of the small cerata, but not in the large ones. Since no other species have been described in such detail and also by extended histology, we can not discuss this feature as typical for the new species.

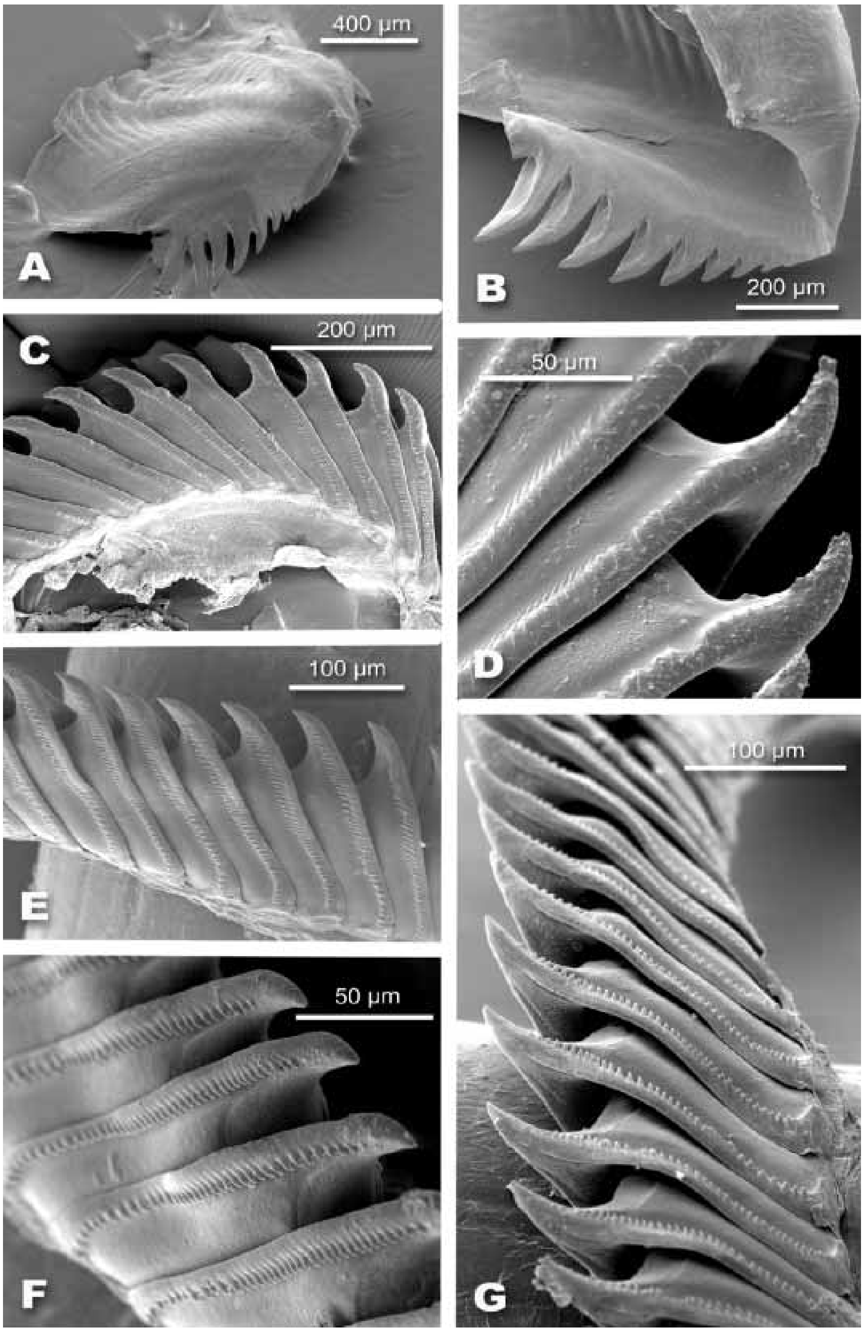

FIGURE 3: *Phyllodesmium jakobsenae*, hard structures in digestive system: A: Right jaw seen from the outside. B: Left jaw from the inside, denticles at the masticatory border seen from the inside. C: Distal part of radula of specimen No. 5. D: Close-up of distal part of radula of specimen No. 5; note the worn denticles on the edge of the rhachidian teeth.. E: Distal part of radula of specimen No. 3. F: Different angle of view of rhachidian cusps from No. 3. G: Part of the radula of specimen No. 3.

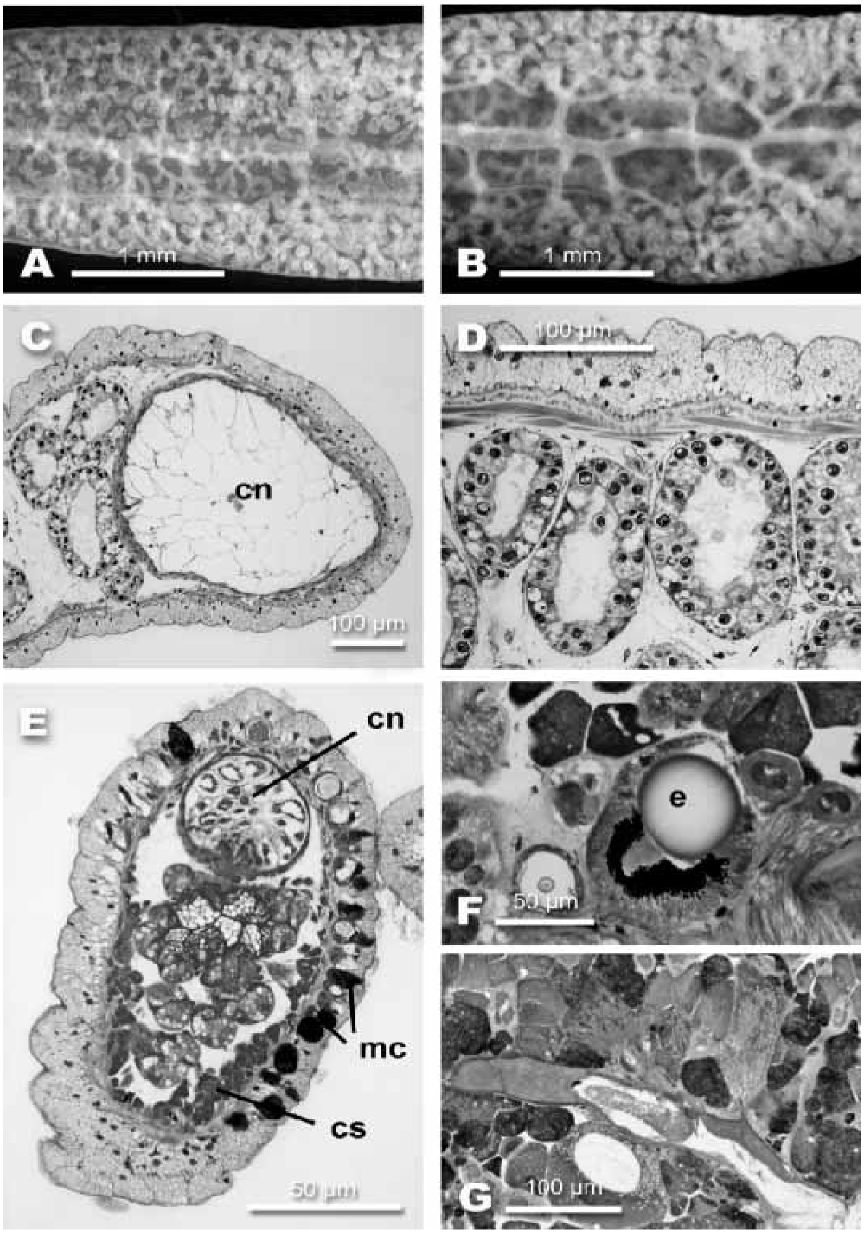

FIGURE 4: *Phyllodesmium jakobsenae*, histology: A: View of ceras from side orientated to light. Note the dense branches along the whole ceras. B: View of ceras from side orientated away from light. Note the main branch of digestive gland and the fewer ramifications. C: Cnidosac of large ceras with no nematocysts. D: Digestive glandular branches beneath epidermis in large ceras. Note the many zooxanthellae especially in the digestive glandular tissue. E: Longitudinal section of small ceras with cnidosac. The digestive glandular duct is not branching, and shows glandular cells. The cnidosac is filled with many tiny nematocysts. The epidermis shows many glandular cells, beneath the epidermis a layer of “cellules spéciales” can be seen. F: Section trough eye and statocyst with one statolith. G: Small part of the oral gland with outleading duct. Abbreviations: cn cnidosac, cs “cellules spéciales”, e eye, mc mucous cells, st statocyst.

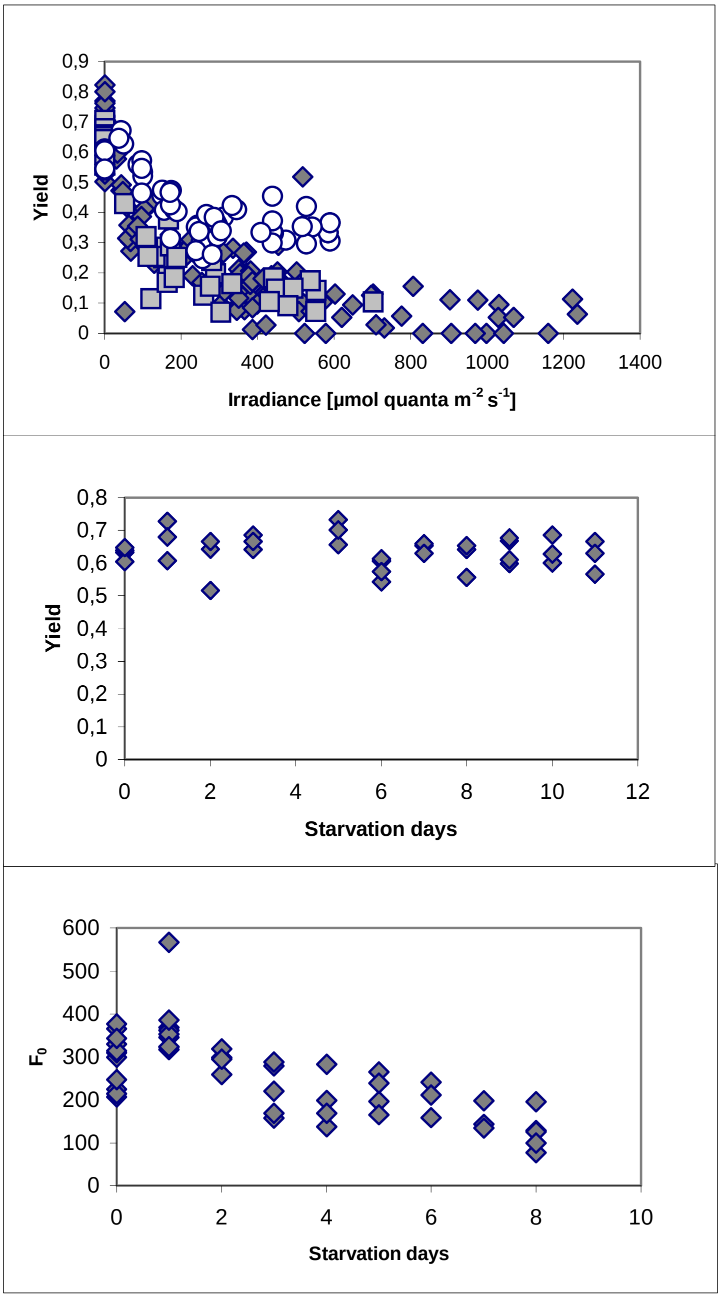

FIGURE 5: Measurements of photosynthetic activity of zooxanthellae *in situ*: A: Yield values measured against irradiance (light intensity) of *Phyllodesmium jakobsenae*, *P. crypticum* and *Xenia* sp. Note the higher values for *Xenia* at same irradiances. B: Yield values of *P. jakobsenae* measured over time during starvation period. High values are maintained during the whole time, indicating healthy zooxanthellae. C: Groundfluorescence of *P. jakobsenae* during starvation. Decline of values indicates the decrease of numbers of zooxanthellae.

◆ *P. jakobsenae*, ■ *P. crypticum*, ○ *Xenia* sp.

Discussion of mutualistic symbiosis

Three *Xenia*-feeding species of *Phyllodesmium* have been found during our expedition to Northern Sulawesi: *Phyllodesmium jabosenae,* one more undescribed species of *Phyllodesmium* with rounded cerata, mimicking a whole tentacle of *Xenia* (also Pulau Bunaken and additionally close to Pulau Talise) and *P. crypticum* from Lembeh Strait (North Sulawesi).

Except for the hitherto undescribed two species, all *Xenia* feeding species (*P. crypticum*, *P. pecten* and *P. hyalinum*) are already known to have a symbiotic relationship with zooxanthellae (Rudman 1981, 1991). The dorso-ventrally flattened cerata increase the surface area exposed to light available to the zooxanthellae. Rudman (1991) postulated an evolutionary history of the symbiotic relationship within the genus *Phyllodesmium*, based on branching patterns of the digestive gland. According to him a higher branching grade of the digestive gland is correlated with a more effective symbiosis. In contrast to the assumption of Rudman (1991) that all *Xenia* feeding species represent an early stage in the development of zooxanthellae symbiosis with a short-term retention of zooxanthellae, *P. jakobsenae* shows a high grade of branching (secondary, according to the terminology of Rudman 1981, 1991) of the digestive gland, similar to *P. hyalinum*, but with sac-like structures for storing zooxanthellae at the end of the fine branches. These sac-like structures are mainly situated in the dorsal, light-exposed side of the ceras and suggest a highly evolved mutualistic relationship with zooxanthellae as can be seen in *P. macphersonae, P. briareum* and *P. colemani* ñ species that feed on other soft corals. Compared to these species *Phyllodesmium jakobsenae* has similar flattened cerata and, compared to other *Xenia* feeding species, the cerata are more flattened. According to Rudman (1991) this indicates an adaptation to the symbiosis.

As already suggested by Rudman (1991) for *Xenia* feeding species (*P. crypticum*, *P. pecten* and *P. hyalinum*), we assume that housing zooxanthellae has advantages for *P. jakobsenae*. Primarily it is color camouflage. The cerata mimicking the polyps of the coral indicate crypsis as an important evolutionary trait.

The availability of additional nutrients (photosynthetic products) is a second advantage.

The fact that all *Phyllodesmium* species associated with *Xenia* are only found on their food coral does not necessarily prove that they still depend on their food source (Rudman 1991). Other cryptic species that are known to have a highly evolved symbiosis (e.g. *P. briareum*; Rudman 1991) are also mainly observed on their food coral (unpublished data). The availability of these additional nutrients may allow the animals to reduce uptake of coral tissue to a minimum, and therefore spare and save their “home” coral.

Histological sections show intact zooxanthellae inside the digestive glandular cells and the lumen of the branches of *P. jakobsenae* (Fig. 4D). Measurements with a Diving-PAM indicate that these dinoflagellate cells are alive and have a photosynthecic activity within *P. jakobsenae* (Figs. 5A–C). The conditions under which the animals were kept were nearly constant for the whole time of the experiments, but not very good: Water filters in the aquaria were not available and the water temperature was too high. Additionally, the animals were sometimes exposed to direct sunlight during taking measurements in high irradiances with the PAM. Nevertheless, the yield in *P. jakobsenae* stayed on a high level for the whole time, even after 12 days of starvation (Fig. 5B). We can assume that the symbiotic relationship between this species and the involved zooxanthellae is stable at least for some days and there is no high turnover of zooxanthellae like suggested for the other *Xenia* feeding species (Rudman 1991). But the decrease of the ground fluorescence values (F_0) in time (Fig. 5C) strongly indicates that the number of zooxanthellae declines (G. Johnsen, pers. comm. 2004). At the same time the animals shrunk and autotomized cerata. Therefore loss of zooxanthellae is considered to be a consequence of the suboptimal cultivation conditions rather than of ongoing digestion.

The decrease of the yield values plotted versus irradiance (Fig. 5A) give an evidence for the acclimatisation of zooxanthellae to light intensities. The photosynthetic characteristics of *P. jakobsenae* are very similar to the ones of *P. crypticum*, although this species was collected from different localities. This indicates that both species house individuals of the same *Symbiodinium* species or clade, with similar ecophysiology. The yield values of *Xenia* are somewhat higher than the ones of the two *Phyllodesmium* species. Therefore we assume that additional ecotypes, strains or even species of *Symbiodinium* that are adapted to higher irradiances are present inside *Xenia*. These additional ecotypes are probably lacking in both *Phyllodesmium* species. A selective uptake or different physiological properties of the various *Symbiodinium* clades, which regulate the incorporation in the slugs, might be the explanation for this phenomenon.

Further studies on the photosynthetic performance of zooxanthellae with different species of *Phyllodesmium* are already performed or will be done in future and will elucidate the role of symbiosis in the evolutionary history of this taxon. Additionally genetic investigations on the involved zooxanthellae will clarify the biodiversity of the involved *Symbiodinium* and their influence on the ecology and physiology of this symbiosis.

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