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Antithamnion nipponicum (Ceramiaceae, Rhodophyta), incorrectly known as *A. pectinatum* in western Europe, is a recent introduction along the North Carolina and Pacific coasts of North America

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Female and male reproductive structures in material from California known as *Antithamnion hubbsii* are illustrated and described for the first time. This partly prostrate species is characterized vegetatively by pinnae arranged in distichous opposite pairs, bearing adaxial and abaxial pinnules, with the distal-most pinnules restricted to the abaxial side of pinnae. Basal cells of the pinnae produce multicellular rhizoids with digitate holdfasts as well as indeterminate lateral axes, and gland cells originate adaxially alongside the lower pinnule cells. *Antithamnion nipponicum* has been placed in synonymy with *Antithamnion pectinatum*, a species described from Auckland I., New Zealand. The latter is here recognized as a separate southern hemisphere species bearing adaxial and abaxial distal-most pinnules and new indeterminate lateral axes in place of pinnae. The correct name for the invasive species known in western Europe as *A. pectinatum* is *A. nipponicum*. Our phylogenetic analyses of *rbc*L sequence data also indicate that Californian *A. hubbsii* and *A. nipponicum* are conspecific, but distinct from *A. pectinatum* and *A. aglandum*. The distribution of *A. nipponicum* includes the Pacific coast of California and the Atlantic coast of North Carolina, USA. Its presence is inferred in the Mediterranean Sea. Historical reports suggest that this species was recently introduced from Japan.

Key words: *Antithamnion*, *Antithamnionella*, *Antithamnion hubbsii*, *A. nipponicum*, *A. pectinatum*, Atlantic Ocean, Antithamnieae, California, Ceramiaceae, invasive species, morphology, North Carolina, *rbc*L, Rhodophyta, taxonomy

Introduction

The circumscription of the genus *Antithamnion* Nägeli (1847) in the tribe Antithamnieae Hommersand (1963) has been modified several times. Feldmann-Mazoyer's (1941) broad definition of the genus included *Pterothamnion* Nägeli, *Antithamnionella* Lyle and *Platythamnion* J. Agardh. Wollaston (1968, 1971, 1972) restricted the genus *Antithamnion* to those species with axes completely lacking rhizoidal cortication, determinate pinnae arranged in opposite pairs, presence of a small quadrate cell at the base of each pinna, presence or absence of gland cells, tetrasporangia cruciately divided, and carpogonial branches borne singly on the basal cells of the pinnae. Maggs & Hommersand (1993) further described in great detail the pre- and post-fertilization reproductive morphology that characterizes the genus and

the tribe. The branching pattern of the pinnae and the position of gland cells are useful characters for discriminating between species (e.g., L'Hardy-Halos, 1968; Athanasiadis, 1996).

Antithamnion hubbsii Dawson was described from vegetative material collected in Melpomene Cove, Isla Guadalupe, Baja California, Mexico (Dawson, 1962, p. 16, pl. 5, fig. 2; pl. 6, fig. 3) (holotype: Dawson No. 8302, LAM 500043). A fragment of the holotype and a lateral indeterminate branch (axis) were photographed by Athanasiadis (1996, p. 146, fig. 66*A*, *B*). Previously the only reproductive structures recorded were tetrasporangia (Young, 1981), although recently we collected thalli of *A. hubbsii* from California exhibiting all reproductive stages. *Antithamnion hubbsii* has been reported from California, USA (Abbott & Hollenberg, 1976), Japan (Itono, 1969), south-east South Africa (Norris, 1987), and New Zealand (Adams, 1994, p. 243), and the taxonomy of this species is still confused (Athanasiadis, 1996). Recent collections of a hitherto unreported

Antithamnion species from Atlantic North Carolina, USA, confirmed that, on the basis of thallus morphology and *rbc*L sequence, the Californian and North Carolinian specimens were conspecific.

A review of the literature indicated that species of *Antithamnion* with creeping filaments that grow attached to the substratum by means of digitate multicellular rhizoids, and have distichous pinnae bearing gland cells, encompass several names. Such species have typically been referred to as *A. hubbsii* in the eastern Pacific (Baja California and Pacific Mexico: Dawson, 1962; California: Abbott & Hollenberg, 1976; Young, 1981) and as *Antithamnion nipponicum* Yamada *et* Inagaki in the western Pacific (Japan: Yoshida *et al.*, 1985; Yoshida, 1998; Kamiya & Kawai, 2002; Mine *et al.*, 2003; Korea: Kang, 1966; Lee & West, 1980; Lee *et al.*, 2001). Examining reproductive material of *A. nipponicum* from the vicinity of Otaru, Japan, Abbott (1999) concluded that certain *Antithamnion* collections from Hawaii were *A. nipponicum*. Kim *et al.* (1996) described a closely related species, *Antithamnion aglandum* Kim *et* Lee for specimens from Cheju Island, Korea, which lacked gland cells and produced hair cells in female gametophytes, while Lee *et al.* (2001) suggested that the species referred to as *A. nipponicum* by Lee & West (1980) was actually *A. aglandum*.

Although Itono (1969) referred to the Japanese material he studied as *A. hubbsii*, he expressed doubt that *A. hubbsii* and *A. nipponicum* were distinct species because Dawson's (1962, p. 10) critical character for discriminating between the two species was not clear-cut; i.e., secondary determinate branchlets in *A. nipponicum* were predominantly pectinate or secund, whereas they were predominantly pinnate or forked in *A. hubbsii*.

Athanasiadis & Tittley (1994) and Athanasiadis (1996, p. 147) placed *A. nipponicum* in synonymy (with a question mark) with *Antithamnion pectinatum* (Montagne) Brauner, a species described from Auckland I., New Zealand. Athanasiadis (1996, p. 146) recognized *A. hubbsii* as a species distinct from *A. pectinatum* on the basis of the regular development of new indeterminate lateral axes originating from basal cells of sparsely branched pinnae in the former, while new axes replace a pinna in a pair in the latter. Wollaston & Womersley (1998) dropped the term whorl-branches for the Antithamnieae, using the older term pinna (pinnae) for the opposite determinate laterals and the term pinnule (pinnules) for the branchlets borne on the pinnae; their revised terminology is used here.

Prior to Athanasidis & Tittley's (1994) merger of *A. nipponicum* into *A. pectinatum*, an invasive *Antithamnion* species in the Mediterranean was first recorded as *A. nipponicum* (Verlaque & Riouall, 1989), and subsequently, the name was changed to *A. pectinatum*, a taxon that has since been reported to have spread further in western Europe (Curiel *et al.*, 1996, 1998, 2002; Verlaque, 2001; Boudouresque & Verlaque, 2002). *Antithamnion pectinatum* was recently recorded from the US Atlantic coast off Connecticut (Athanasiadis & Tittley, 1994) and from Australia (Womersley, 1998, p. 106). Adams (1994) did not mention *A. pectinatum* or *Callithamnion pectinatum* Montagne, but instead used the name *Antithamnion applicitum* (Harvey) J. Agardh to refer to all the samples she studied from New Zealand, referring to a smaller plant from the warmer waters of the Kermadec Islands with "gland cells, very conspicuous, sessile on short pinnule" as *A. hubbsii*. Neither species were critically described and no comparisons are possible based on her descriptions (Hommersand, pers. comm.). Norris's (1987) depiction of a species he referred to as *A. hubbsii* from Natal, South Africa, includes minute glandless specimens that clearly represent another species, distinct from both *A. nipponicum* and *A. pectinatum*.

This paper explores the suggestion that specimens from California and Western Europe known as *A. hubbsii* or *A. pectinatum* are really *A. nipponicum*, and that *A. pectinatum* from New Zealand is a distinct species. As an invasive species, the distribution of *A. nipponicum* has extended into North Carolina. We briefly comment on the distribution of *A. nipponicum* vis-à-vis that of *Antithamnionella spirographidis* (Schiffer) Wollaston of the tribe Heterothamnieae.

Materials and methods

Molecular study

DNA samples were prepared using the DNeasy Plant Mini Kit (QIAGEN, Valencia, CA, USA) from fresh, field-collected silica-gel dried specimens. The *rbc*L gene was amplified using the primer combinations: F7-R753 and F645-RrbcSstart as listed in (Lin *et al.*, 2001). Sequencing primers used were: F7, F645, F993, R367, R753, R1150, RrbcS start (Freshwater & Rueness, 1994; Lin *et al.*, 2001; Gavio & Fredericq, 2002). Amplification conditions followed Cho *et al.* (2003). Sequences were determined for both forward and reverse strands using an ABI Prism 3100 Genetic Analyzer (PE Applied Biosystems, Foster City, CA) with the ABI Prism BigDye™ Terminator Cycle Sequencing Ready Reaction Kit (PE Applied Biosystems). Silica gel-dried specimens and extracted DNA samples are deposited at the University of Louisiana at Lafayette, stored at −20°C. Seven taxa representing four species of Antithamnieae, and four taxa representing two species of Heterothamnieae, were sequenced for inclusion in the

phylogenetic analysis (Fig. 1); *Ceramium californicum* J. Agardh of the Ceramieae was used as the outgroup based on phylogenetic hypotheses derived from earlier global analyses of the Ceramiaceae (data not shown). DNA sequences have been deposited in GenBank. GenBank accession numbers, species identification and authors, information concerning origin, date and collectors are listed in Table 1.

The generated *rbc*L sequence data were compiled and manually aligned with Sequencher (Gene Codes Corp., Ann Arbor, MI) and MacClade 4.0 (Maddison & Maddison, 2000) and exported for phylogenetic analysis. Phylogenetic analyses were conducted with Maximum Likelihood (ML) and Maximum Parsimony (MP) algorithms as implemented in PAUP*v.4.0 beta 10 (Swofford, 2002). Only the ML tree is shown here (Fig. 1), as the tree topology was similar to the MP tree. For the ML analyses, the aligned sequences were first analyzed with Modeltest (v.3.0, Posada & Crandall, 1998), which compared different models of DNA substitutions in a hierarchical hypothesis-testing framework, to select a base substitution model that best fits our sequence data. The optimal model for the sequence was a GTR (General Time Reversible model, Rodriguez *et al.*, 1990) + G (Gamma distribution). The parameters were as follows: assumed nucleotide frequencies A = 0.3139, C = 0.1569, G = 0.2096, T = 0.3196; substitution rate matrix with A–C substitutions = 1.5763, A–G = 4.0321, A–T = 4.1766, C–G = 0.9943, C–T = 13.4581, G–T = 1.0000; proportion of sites assumed to be invariable = 0; gamma distribution shape parameter = 0.2024. The ML tree was generated by a heuristic search of 1,000 random additions holding 1 tree at each step under the invoked settings for the respective base substitution model. Support for nodes was determined by calculating bootstrapping proportion

values (Felsenstein, 1985) using 1,000 bootstrap replicates for the ML analysis. MP trees were inferred from a heuristic search and option support for nodes was determined by calculating bootstrapping proportion values (Felsenstein, 1985) using 1,000 replicates for MP analyses

Morphological study

Details of the specimens examined and their collection sites are given in Table 1. Material was preserved in silica gel upon collection in the field and subsequently transferred to 5% formalin/seawater, or immediately submerged in 5% formalin/seawater. Microscopic observations were made from material stained with 1% aqueous aniline blue, but two minute fragments of the holotype of *A. pectinatum* from Auckland, New Zealand were not stained. Vouchers are deposited in LAF (herbarium abbreviations follow Holmgren *et al.*, 1990). Photomicrographs were taken with a Polaroid DMC Ie digital camera (Polaroid, Inc., Cambridge, MA) attached to an Olympus BX60 (Olympus, Melville, NY). Images were edited and assembled into plates using *Photoshop* v.5.0 (Adobe Systems Inc., San Jose, CA). A total of 15 individuals of *A. nipponicum* (including *A. hubbsii*) were selected for quantitative measurements. *Antithamnionella spirographidis* was collected from the Pacific and Atlantic.

Results

Molecular analyses

*Rbc*L sequences of *A. nipponicum* were generated from four samples: one from California
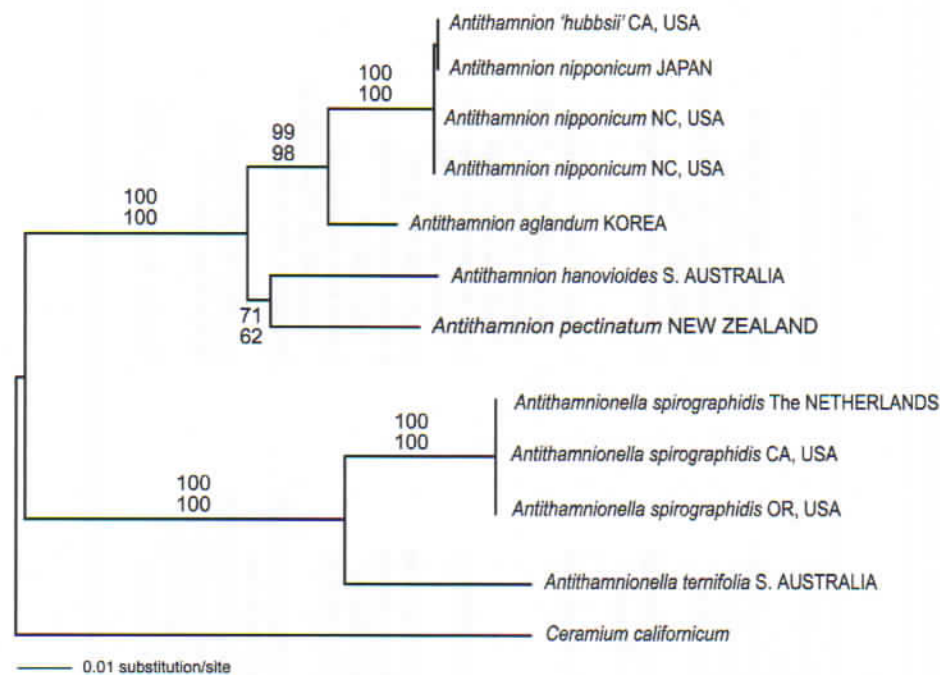

Fig. 1. Maximum likelihood tree inferred from 11 *rbc*L sequences of *Antithamnion* and *Antithamnionella* species, and from one specimen of *Ceramium californicum* used as the outgroup. The Ln likelihood of the tree was −3992.6321. Bootstrap values (>50%) are shown for all ML (top) and MP (bottom) analyses.

Table 1. Taxon and collection information for specimens used in the morphological study and *rbc*L analyses

Species	Location	Collection date	Collector	Habit/attachment	GenBank number
Antithamnion aglandum Kim *et* Lee	Jeongdori, Wando, Korea	29.Jan.1999	S.M. Boo, T.O. Cho & H.G. Choi	Creeping on rope	AY594700
Antithamnion hanovioides (Sonder) De Toni	Pennington Bay, Kangaroo Island, S. Australia	7.Sept.1995	M. Hommersand		AY591927
Antithamnion 'hubbsii' Dawson	Halfmoon Bay, San Mateo Co., California, USA	3.Jun.2003	T.O. Cho & B.Y. Won	Creeping on decaying wood of floating docks	AY591930
Antithamnion nipponicum Yamada *et* Inagaki	Hachinohe, Aomori, Japan	21.May.1995	M. Kamiya	Culture strain #1078, Kobe University Research Center for inland seas	AY594699
Antithamnion nipponicum	In front of Duke University Marine Laboratory, Pivers I., Beaufort, Carteret Co., North Carolina, USA	29.Oct.2003	T.O. Cho & B.Y. Won	Creeping on *Hypnea* sp.	AY591928
Antithamnion nipponicum	Jetty, Beaufort, Carteret Co., North Carolina, USA	29.Oct.2003	T.O. Cho	Creeping on *Hypnea* sp.	AY591929
Antithamnion nipponicum	Torumi, Kobe, Japan	15.Jul.2004	S. Kawaguchi		DQ002481
Antithamnion pectinatum (Montagne) Brauner	Lee Bay, Stewart Island, New Zealand	3.Oct.2004	W.A. Nelson	Creeping on *Laurencia thyrsifera*	
Antithamnion pectinatum	Island Bay, Wellington, North Island, New Zealand	22.Aug.1990	W.A. Nelson	Creeping on *Gelidium* sp.	-
Antithamnionella spirographidis (Schiffner) Wollaston	Burghsluis, Netherlands	8.Aug.1997	M. Hommersand		AY591925
Antithamnionella spirographidis	Monterey Bay, California	11.Dec.1999	T.O. Cho		AY591923
Antithamnionella ternifolia (Hooker *et* Harvey) Lyle	Williamston, Australia	28.Mar.2002	J. West		AY591926
Ceramium californicum J. Agardh	Yaquina Bay, Oregon, USA	13.Jul.1998	T.O. Cho & G.-I. Hansen		AY591931

(*A. 'hubbsii'*), two from North Carolina, and one from Japan. Of the 1,429-bp portion analyzed (99% sequenced), 1,135 bp are constant, 102 are variable, and 192 are phylogenetically informative. The *rbc*L sequences between the Pacific (California) and Atlantic (North Carolina) vouchers differed at only one of the sequenced 1,460 sites (position 835: a T in Pacific vs G in Atlantic specimens), whereas the sequences between the individuals from California and Japan were identical. *Antithamnion nipponicum* differed from *A. pectinatum* by 5.4–5.5% sequence divergence, from *A. aglandum* by 2.7% sequence divergence, and from *A. hanovioides* by 5.7–5.8% sequence divergence.

Antithamnion formed a distinct clade separated from *Antithamnionella* with strong bootstrap support (Fig. 1) in both ML and MP trees. All samples of *A. 'hubbsii'* and *A. nipponicum* were also placed in a strongly supported (100% for ML, 100% for MP) monophyletic clade and clearly separated from *A. pectinatum*, *A. aglandum* and *A. hanovioides*. The *A. spirographidis* sequences from the Atlantic (the Netherlands) and Pacific (Oregon and California) oceans were identical, and all sequenced populations fall in a strongly supported (100% for ML and MP) monophyletic clade.

Morphological observations

Vegetative morphology of A. nipponicum*:* The thallus is composed of prostrate and erect axes (Fig. 31) with the prostrate axis consisting of axial segments growing parallel to the substratum, as shown in the North Carolina material. In vouchers from other localities, the prostrate axes were missing from the collecting protocol. We were not able to determine whether the prostrate axes are erect axes that have become secondarily attached to the substratum. The erect axis comprises main and indeterminate lateral axes of unlimited growth bearing opposite pinnae of determinate growth, which in turn bear distichous-opposite pinnules (Fig. 2). Indeterminate lateral axes are irregularly produced from the basal cell of pinnae in erect axes (Figs 6, 35).

Apical growth proceeds through transverse divisions of terminal cells producing a straight-to-slightly curved apex (Figs 3, 32). Apical cells are 4–5 µm in diameter and 1.1–1.3 times longer than broad, and subterminal axial cells increase in size away from the apex to 270–340 µm in diameter and 3.5–4.2 times longer than broad. Each axial cell produces a pair of lateral initials on either side; a lateral initial is cut off by oblique longitudinal division on one side and then the other, followed by the initial on the opposite side (Fig. 3), resulting in a pair of upwardly curved opposite pinnae (Fig. 32).

Pinnae are 550–600 µm long, composed of 11–13 cylindrical rachis cells on a quadrangular basal cell. Rachis cells are each 50–70 µm in diameter and 3–3.5 times longer than broad, and the filament (pinna) tapers to an acute apical cell. At maturity, the pinnae on the upper part of each axial cell are distichous-opposite at 45° to the main axis (Figs 4, 5, 33). The basal cell of a pinna is small, about 12–13 µm long and 10–11 µm in diameter, typically subspherical or quadrate in shape (Figs 6, 34), and can initiate rhizoids as well as indeterminate lateral axes (Figs 6, 9).

Pinnules can be produced on the adaxial and abaxial sides of each pinna rachis cell up to the first seven cells. The first pinnules are produced abaxially and the adaxial pinnules form later on. Six to seven pinnules are produced on the abaxial side, and 3–5 on the adaxial side (Figs 4, 5). Pinnules are 20–25 µm in diameter, 2–3 times longer than broad, 5–8 cells long on the adaxial side, 7–9 cells long on the abaxial side, tapering to a small mucronate apical cell. Although most pinnules are simple, lowermost pinnules can bear secondary branchlets on their abaxial side (Fig. 5).

Gland cells (Figs 7, 8, 34) are common and cut off adaxially by a slight concavo-convex division from the upper part of the basal cells of pinnules, and are subtended by the suprabasal cell (Figs 7, 8). They are ovate-to-oblong, about 20 µm long and 12 µm in diameter, pale green or yellowish, and reach the upper boundary of the next cell. Gland cells from specimens from North Carolina contained small darkly staining bodies (Fig. 34).

The uniseriate rhizoids originate from rhizoid initials cut off abaxially from the basal cells of pinnae and continue to grow towards the substratum at 45° to the pinna (Fig. 9). Three-to-four-celled rhizoids terminate in an irregular multicellular lobed disc (Fig. 9) that attaches to the epiphyte host or substratum.

Reproductive structures of A. nipponicum*:* Tetrasporangia are scattered in the upper and middle thallus region of the tetrasporophyte (Fig. 10). Tetrasporangial initials are produced singly on the adaxial sides of the basal-most pinnule cells and do not develop beyond the third or fourth pinnule cell (Figs 11, 12). Tetrasporangia are cruciately divided, sessile, spherical to ellipsoidal, 60–70 µm long and 45–55 µm in diameter, excluding the sheath (Fig. 13).

Spermatangia develop on spermatangial heads of male gametophytes (Fig. 14). Most spermatangial heads replace pinnules in the upper thallus
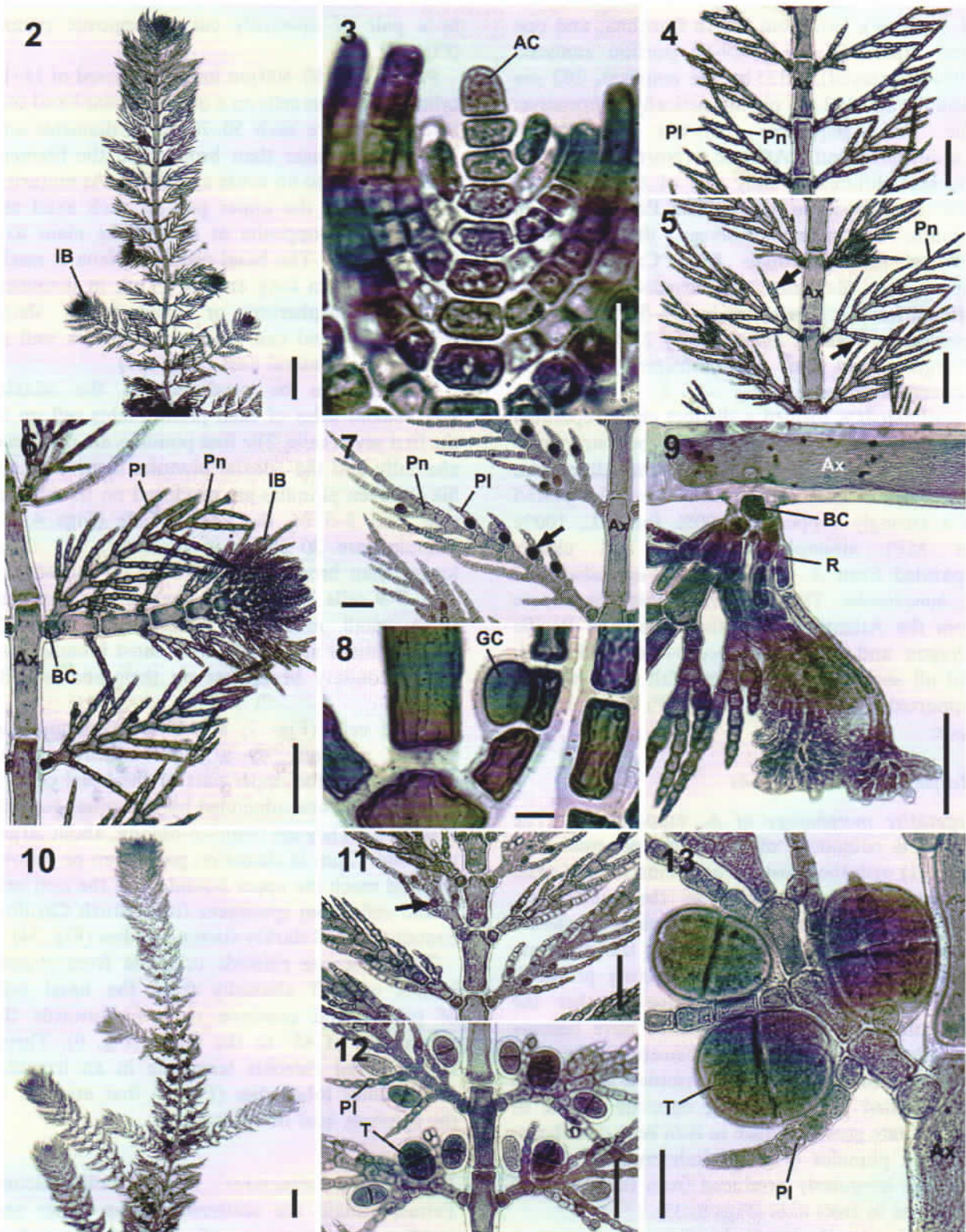
Fig. 2–13. *Antithamnion nipponicum* from Halfmoon Bay, California, USA. Figs 2–9. Vegetative structure. Fig. 2. Upright thallus. Scale bar 0.5 mm. Fig. 3. Apical region showing successive development of opposite pinnae. Scale bar 100 μm. Fig. 4. Pinnae near apex with mostly abaxial pinnules. Scale bar 50 μm. Fig. 5. Pinnae with basal branched pinnules (arrows) in the middle thallus. Scale bar 100 μm. Fig. 6. Indeterminate lateral axis arising from basal cell of pinna. Scale bar 100 μm. Fig. 7. Gland cell (arrow) developing adaxially from lowermost cells of pinnules. Scale bar 50 μm. Fig. 8. Gland cell developing adaxially from upper side of basal cell of pinnule. Scale bar 5 μm. Fig. 9. Rhizoids developing from basal cell of pinna and terminating in multilobed disc. Scale bar 100 μm. Figs 10–13. Tetrasporic structure. Fig. 10. Erect tetrasporangial thallus with tetrasporangia on the main and lateral axes. Scale bar 0.5 mm. Fig. 11. Pinna bearing tetrasporangial initials (arrows). Scale bar 100 μm. Fig. 12. Pinna bearing mature tetrasporangia. Scale bar 100 μm. Fig. 13. Basal-most cells of pinnules bearing tetrasporangia. Scale bar 40 μm. Abbreviations: AC: apical cell; Ax: axial cell; BC: basal cell; GC: gland cell; IB: indeterminate lateral axis; R: rhizoid; T: tetrasporangium; Pl: pinnule; Pn: pinna.
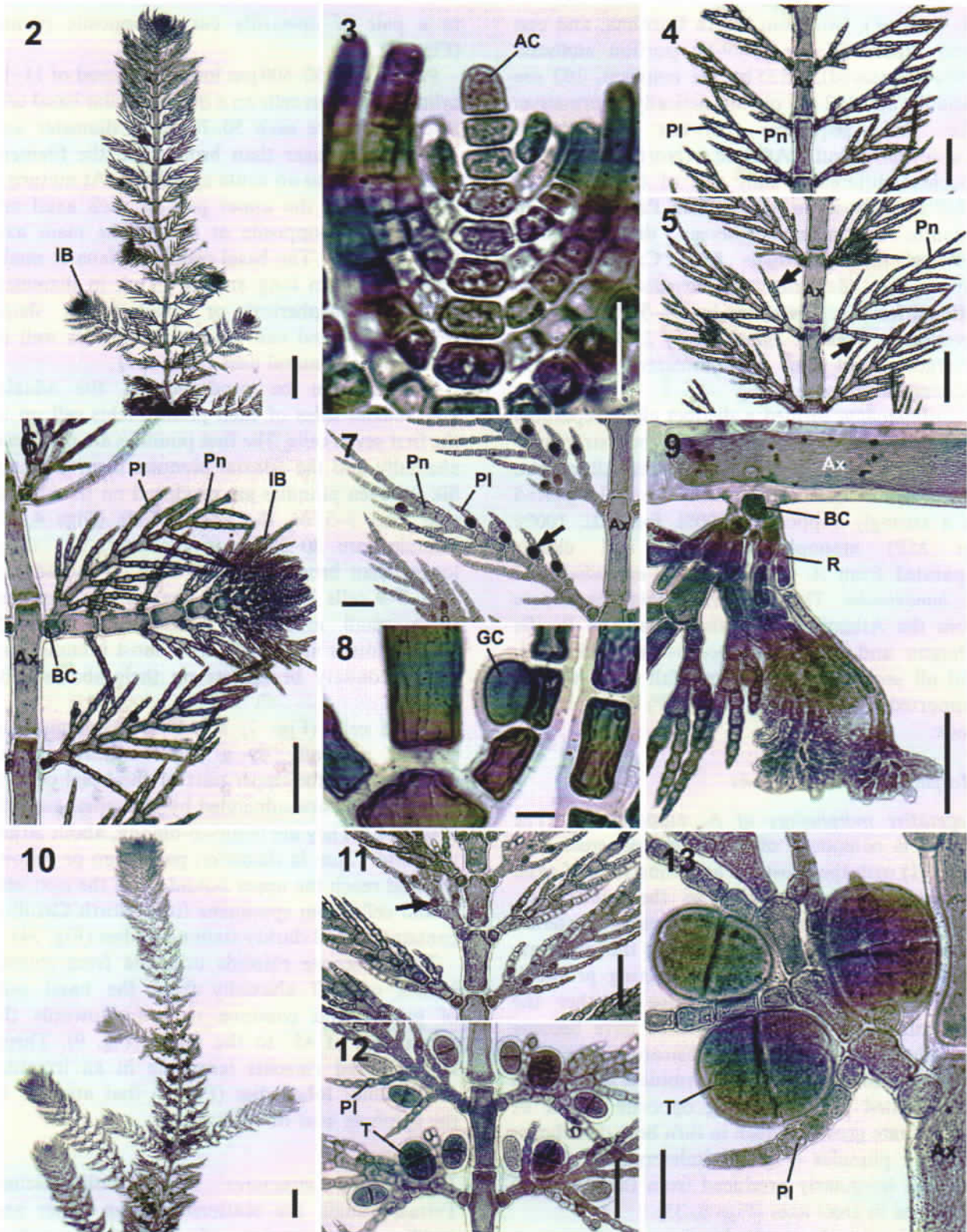
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(Figs 15, 16), while some occur on special short branches on the adaxial side of the pinnules in the lower thallus (Fig. 19). The spermatangial head cells cut off spermatangial parent cells in small clusters from their upper end (Figs 17, 20). Each spermatangial parent cell bears one to two spermatangia (Figs 18, 21). Spermatangia are colourless and elliptical-to-spherical, about 3 μm long and 2.5 μm in diameter.

In female thalli (Fig. 22), young pre- and post-fertilization stages are found in the upper parts of the thallus (Fig. 23). Procarps are formed singly or in pairs on every segment of the main and indeterminate axes (Fig. 24). The basal cell of a pinna becomes the supporting cell of the carpogonial branch (Fig. 25). After presumed

fertilization, the supporting cell enlarges and cuts off an auxiliary cell (Fig. 26). The carpogonium produces a connecting process that fuses to the auxiliary cell (Fig. 26). The auxiliary cell divides into a foot cell and a gonimoblast initial (Fig. 27). The first gonimolobe is cut off terminally from the gonimoblast initial (Fig. 28), followed by the production of one or two additional gonimolobe initials laterally (Fig. 29). The supporting cell, foot cell and axial cell fuse into a fusion cell product (Fig. 29). Mature cystocarps are spherical, about 210–280 μm long and 190–230 μm in diameter (Fig. 30). Morphological features of *A. nipponicum* from Japan, shown in Figs 36–41, conform to the material from California and North Carolina.
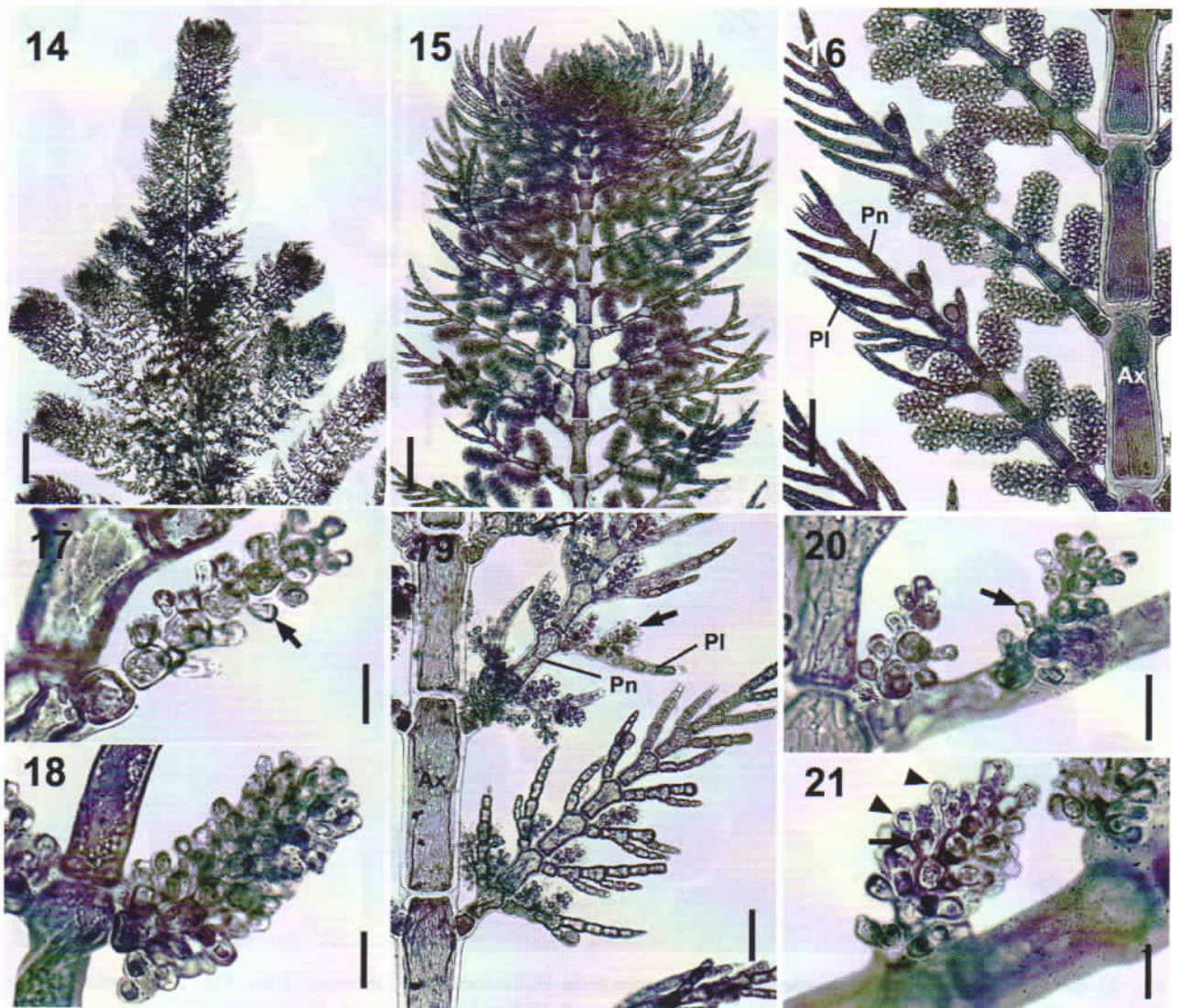

Fig. 14–21. Male structures of *Antithamnion nipponicum* from Halfmoon Bay, California, USA. Fig. 14. Male thallus. Scale bar 0.5 mm. Fig. 15. Apical region of male thallus. Scale bar 200 μm. Fig. 16. Spermatangial heads replacing pinnules. Scale bar 200 μm. Fig. 17. Spermatangial parent cells (arrow) borne on pinnule-like spermatangial head. Scale bar 10 μm. Fig. 18. Mature spermatangia borne on pinnule-like spermatangial head. Scale bar 10 μm. Fig. 19. Spermatangial heads (arrow) produced on special branches on adaxial side of pinnule. Scale bar 50 μm. Fig. 20. Spermatangial parent cells (arrow) produced on special branches on adaxial side of pinnule. Scale bar 10 μm. Fig. 21. Spermatangia (arrowhead) borne on spermatangial parent cells (arrow) on special branches on adaxial side of pinnule. Scale bar 10 μm. Abbreviations as in Figs 2–13.
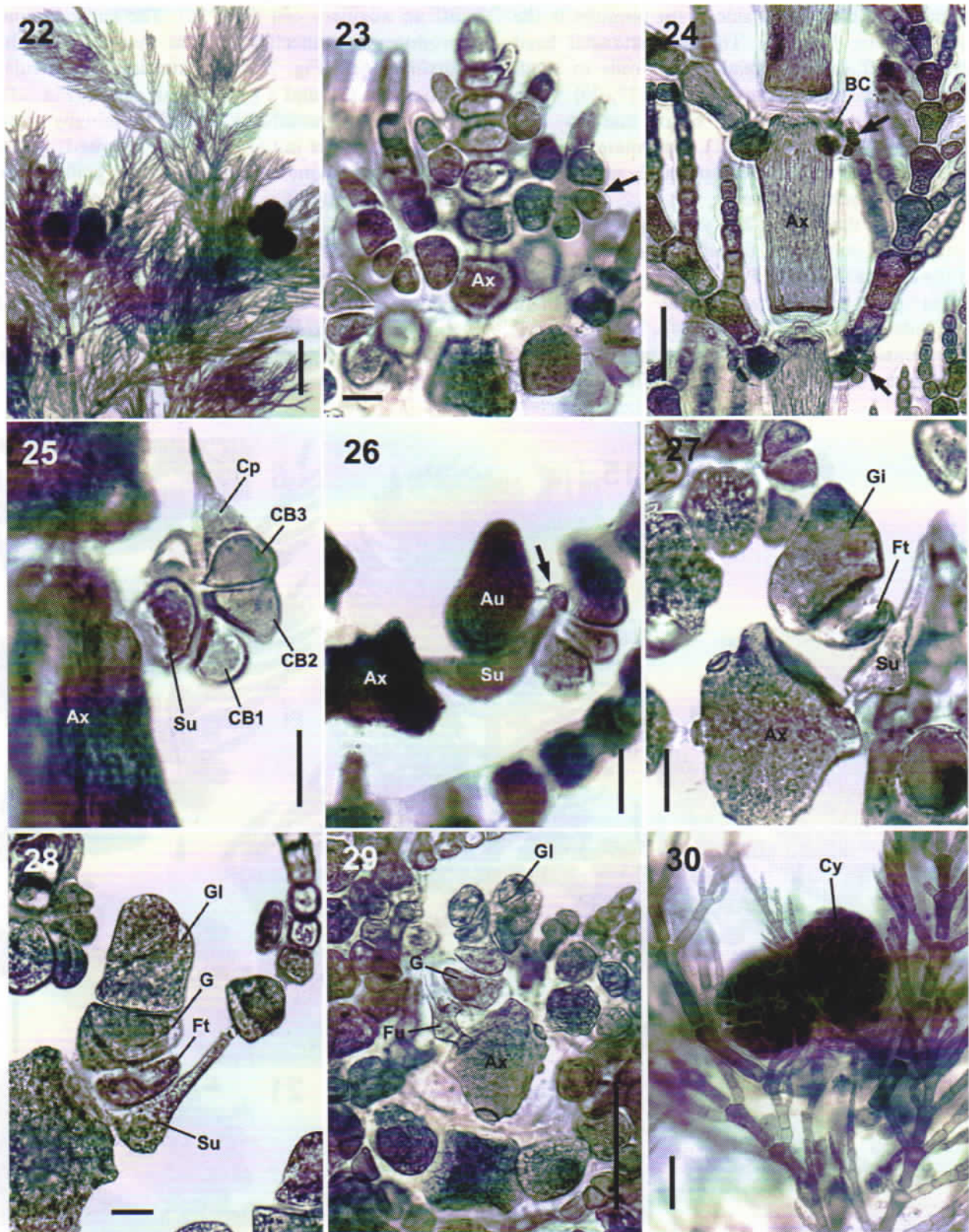
Fig. 22–30. Female structures of *Antithamnion nipponicum* from Halfmoon Bay, California, USA. Fig. 22. Female thallus. Scale bar 250 µm. Fig. 23. Apical region with procarp (arrow). Scale bar 4.5 µm. Fig. 24. Upper part with procarps (arrows) borne on basal cells of pinna. Scale bar 50 µm. Fig. 25. Procarp composed of carpogonial branch and supporting cell. Fig. 26. Post-fertilization stage showing auxiliary cell borne on supporting cell, and connecting process (arrow) emanating from carpogonium and linking to auxiliary cell. Fig. 27. Formation of gonimoblast initial and foot cell from auxiliary cell. Fig. 28. Formation of first gonimolobes from gonimoblast initial. Scale bars 5 µm. Fig. 29. Formation of fusion cell from foot cell and supporting cell. Scale bar 40 µm. Fig. 30. Mature, naked cystocarp with two gonimobles. Scale bar 100 µm. Abbreviations: Au: auxiliary cell; CB1–3: sequence formation of carpogonial branch cells; Cp: carpogonium; Cy: cystocarp; Ft: foot cell; Fu: fusion cell; G: gonimoblast; Gi: gonimoblast initial; Gl: first gonimolobes; Su: supporting cell.

Vegetative morphology of A. pectinatum *and* A. aglandum*:* In type material of *A. pectinatum* (Figs 42–44) indeterminate lateral axes appear to replace pinnae (Fig. 43) and gland cells are located on determinate 3-celled branchlets (Fig. 44). Material of *A. aglandum* (Fig. 45) lacks secondary branchlets (Figs 46, 47) and both indeterminate lateral axes and rhizoids are produced from basal cells of pinnae (Fig. 46).

Discussion

Our phylogenetic analyses indicate that the Californian material known as *A. 'hubbsii'* is conspecific with *A. nipponicum*, but distinct from *A. pectinatum* and *A. aglandum*. The specimens from California and North Carolina correspond to the original description and figures of the type

of *A. hubbsii* (Dawson, 1962: 16, pl. 5, fig 2) and to its interpretation in subsequent work (Athanasiadis, 1996: 146, fig. 66), including habit, branching pattern, and the development of new indeterminate lateral axes. In particular, distal pinnules only occur on the abaxial side of pinnae, indeterminate lateral axes are produced from basal cells of the pinnae, and gland cells occur on lower cells of normal pinnules. Rhizoids and determinate axes developing from basal pinna cells have also been illustrated in *A. nipponicum* from Japan (as *A. hubbsii*, Itono, 1969) and western Europe (Curiel *et al.*, 1996, fig. 4). These specimens of *A. hubbsii* also conform to authentic *A. nipponicum* obtained from culture strains and field collected material from Japan. All reproductive structures conform to those of other published *Antithamnion* species (Wollaston, 1971; Norris, 1987; Lee & West, 1980; Womersley, 1998).
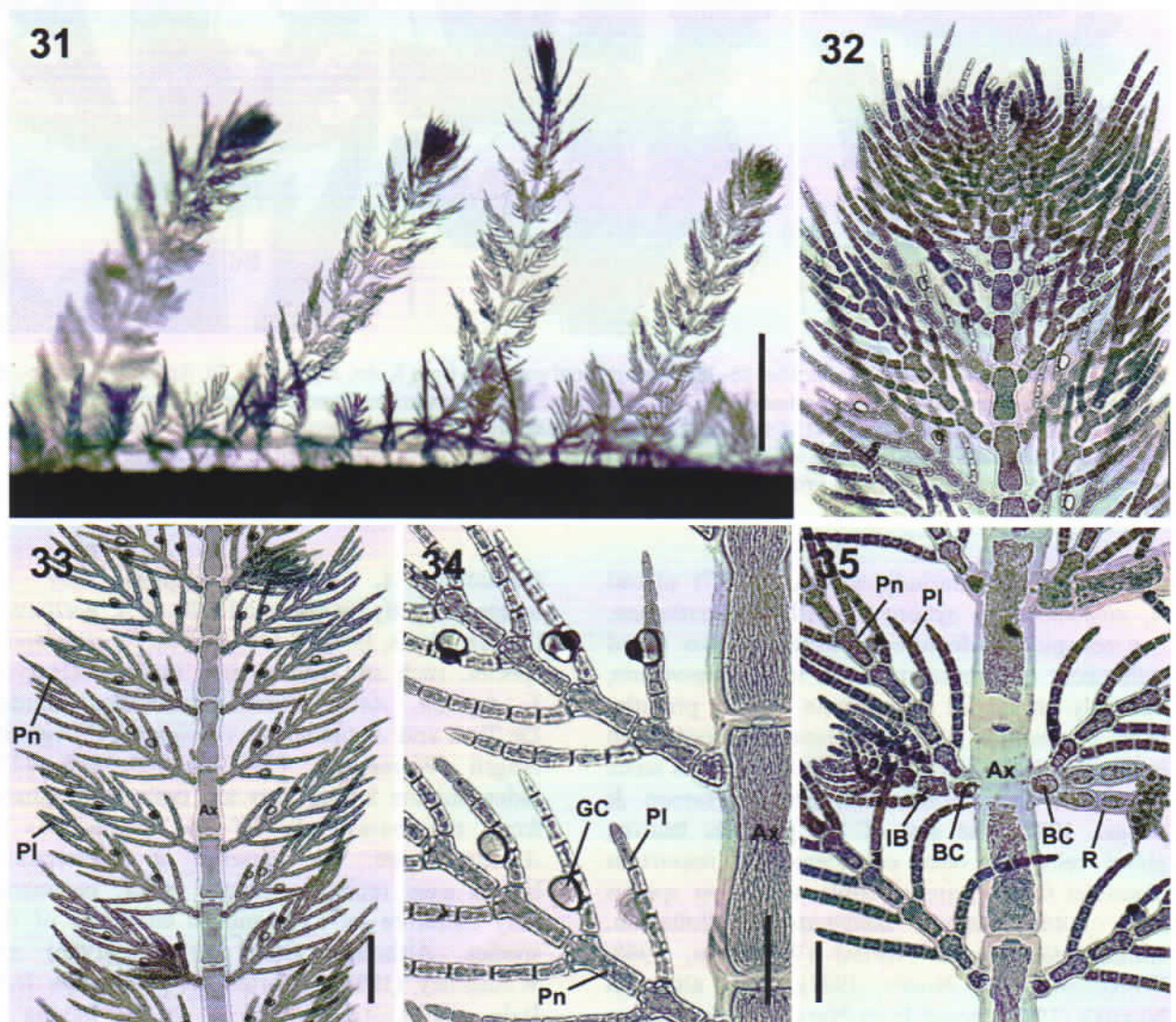

Fig. 31–35. *Antithamnion nipponicum* from Beaufort, North Carolina, USA. Fig. 31. Thallus growing on *Hypnea* sp. Scale bar 500 µm. Fig. 32. Apical region. Scale bar 50 µm. Fig. 33. Pinnae in middle part of thallus. Scale bar 50 µm. Fig. 34. Gland cells. Scale bar 40 µm. Fig. 35. Basal part showing indeterminate lateral axis and rhizoids produced from basal cells of pinnae. Scale bar 50 µm. Abbreviations as in Figs 2–13.
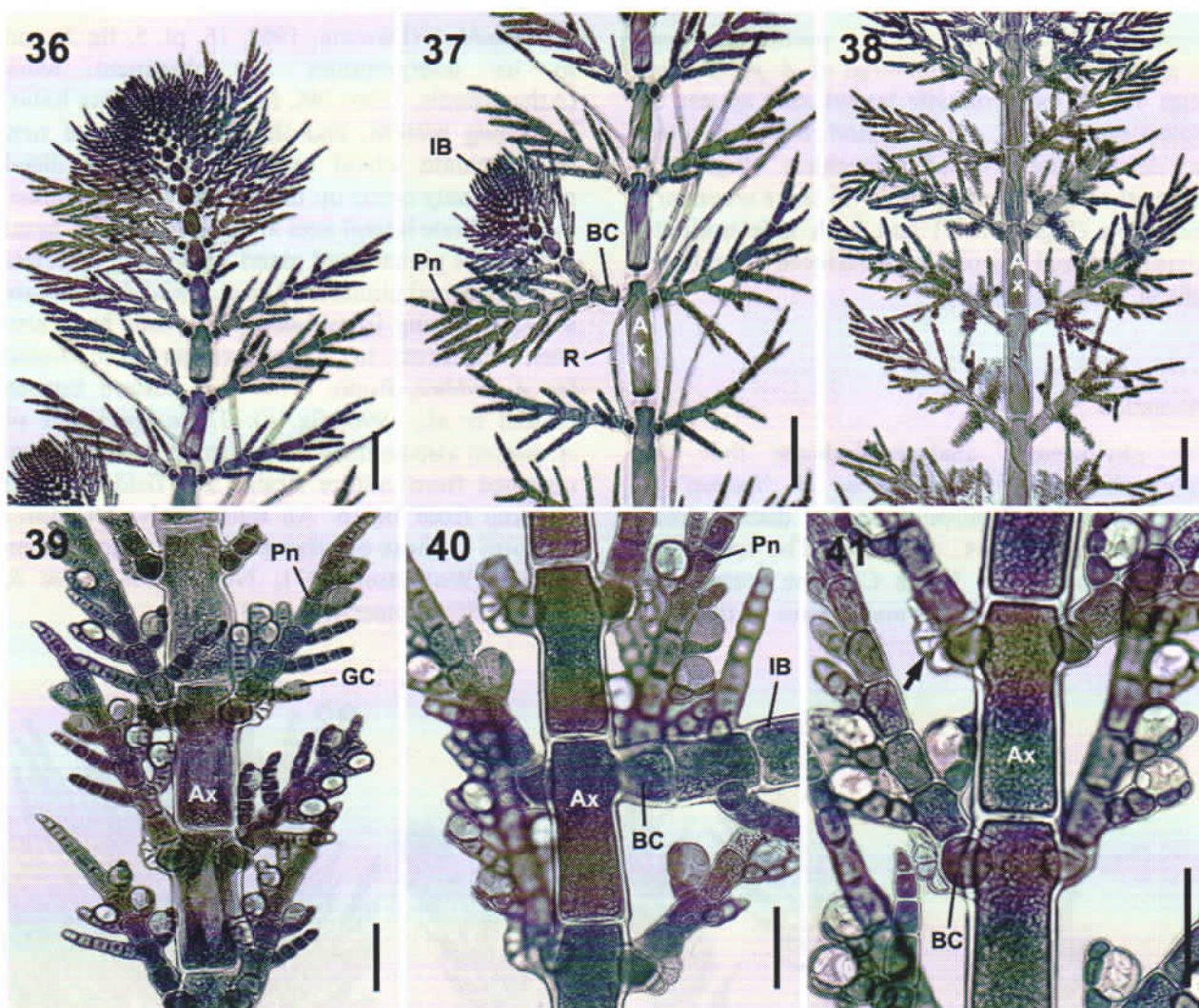
Fig. 36–41. *Antithamnion nipponicum.* Figs 36–38. Field material collected from Kobe, Japan. Fig. 36. Apical region showing successive development of opposite pinnae. Fig. 37. Indeterminate lateral axis arising from basal cell of pinna. Fig. 38. Male thallus. Scale bars 100 µm. Figs 39–41. Female culture strain (strain #1078 in Kobe University Research Center, Japan). Fig. 39. Pinnae. Scale bar 50 µm. Fig. 40. Indeterminate lateral axis produced from basal cell of pinna. Scale bar 40 µm. Fig. 41. Upper part of thallus with procarp born on basal cell (bc). Scale bar 50 µm. Abbreviations as in Figs 2–13.

Although Athanasiadis (1996, p. 147) placed *A. nipponicum* in synonymy with *A. pectinatum*, two conspicuous features separate the two: gland cells occur on normal pinnules in *A. nipponicum*, but only on special determinate 3-celled pinnules in *A. pectinatum*. Although gland cell production is thought to be environmentally induced in some species of the *Antithamnion* group (Hansen & Scagel, 1981), the size of the pinnules bearing gland cells has been considered an important character for the circumscription of other species (e.g., *Antihamnion diminuatum* Wollaston, *Antihamnion eliseae* Norris) (Wollaston, 1968; Wollaston, 1972; Norris, 1987). Also, although Norris's (1987) record from Natal includes minute glandless specimens of *A. nipponicum*, gland cells are observed in the pinnules of all our material. Furthermore, indeterminate lateral axes occur on the basal cells of pinnae in *A. nipponicum*

(including *A.* 'hubbsii') whereas they are located directly on the axial cells in *A. pectinatum* (Athanasiadis, 1996, p. 146). In other *Antithamnion* species, such as *Antithamnion verticale* (Harvey) J. Agardh, *Antithamnion hanovioides* (Sonder) De Toni and *Antithamnion cruciatum* (C. Agardh) Nägeli (Womersley, 1998, p. 105, 110, 117), indeterminate lateral axes are typically produced from the basal cells of the pinnae, as in *A. nipponicum*. The presence of indeterminate lateral axes replacing pinnae in *A. pectinatum* may therefore be a diagnostic character of this species. Although Curiel *et al.* (1996) and Womersley (1998) reported *A. pectinatum* from Italy and Australia, respectively, as having an unlimited number of lateral axes produced from the basal pinna cells, our observation of the type material shows that indeterminate lateral axes are formed directly on the axial cells. After observing

Fig. 42–47. *Antithamnion pectinatum* and *A. aglandum*. Figs 42–44. Fragment of holotype of *A. pectinatum* from New Zealand. Fig. 42. Vegetative thallus. Scale bar 100 μm. Fig. 43. Middle part of thallus showing indeterminate lateral axis replacing a paired pinna. Scale bar 100 μm. Fig 44. Pinnae showing gland cell (arrow) touching three cells. Scale bar 50 μm. Figs 45–47. *A. aglandum* from Korea. Fig. 45. Vegetative thallus. Scale bar 300 μm. Fig. 46. Middle part of thallus showing indeterminate lateral axis (arrow) and rhizoid (arrowhead) produced from basal cell of pinnae. Scale bar 50 μm. Fig. 47. Pinnae. Scale bar 100 μm. Abbreviations as in Figs 2–13.

the holotype, Athanasiadis (1996, p. 146) also separated *A. nipponicum* (as *A. 'hubbsii'*) from *A. pectinatum* based on: (i) the regular development of new indeterminate lateral axes from the basal cells of pinnae, and (ii) sparsely ramified pinnae.

The molecular and morphological evidence strongly suggest that *A. hubbsii* from California is conspecific with *A. nipponicum* and distinct from *A. pectinatum* and *A. aglandum*.

Taxonomic conclusion

Antithamnion nipponicum Yamada & Inagaki, 1935, p. 38, figs 1–3; type locality: Natudomari-zaki, Mutu Bay, Osyoro, Siribesi Prov., Japan; type not designated.

SYNONYM: *Antithamnion pectinatum sensu* Curiel *et al.* (1996).

PROVISIONAL SYNONYM: *Antithamnion hubbsii* Dawson, 1962, p. 16, pl. 5, fig. 2; pl., fig. 3; type locality: Melpomene Cove, Isla Guadelupe, Baja California, Mexico.

HOLOTYPE: Dawson. no. 8302, LAM 5000043, reproduced in Athanasiadis, 1996, fig. 66.

Antithamnion nipponicum has been reported from Saghalien to Korea (Tokida, 1954; Kang, 1966) and Japan (Itono, 1969) (western Pacific), and California (Abbott & Hollenberg, 1976) (eastern Pacific), but also from South Africa (Norris, 1987). This paper shows that its distribution extends to North Carolina, Atlantic, USA. Verlaque & Riouall (1989) reported *A. nipponicum* as an

invasive species in the Mediterranean. After Athanasiadis (1996) maintained *A. hubbsii* as a distinct species but placed *A. nipponicum* into synonymy with *A. pectinatum*, all reports of an invasive *Antithamnion* in western Europe were given as *A. pectinatum*, especially in the lagoon of Venice (e.g., Curiel *et al.*, 1996, 1998, 2002). Since the mid 1980s (Athanasiadis & Tittley, 1994), *A. pectinatum* has also been recorded from the western Atlantic (Connecticut, by R. Wilce), about the same time that invasive *Codium* Stackhouse was reported in North Carolina (USA) by Searles *et al.* (1984). We agree with Verlaque and Riouall (1989) that the Mediterranean species should be designated as *A. nipponicum*, as for the Californian, North Carolinian, and western European taxon. We suggest that *A. nipponicum* became established in central California, the Mediterranean and Adriatic Seas, and the Atlantic Ocean via recent introduction from Japan. The introductions may correlate with the introduction of *Codium fragile* subs. *tomentosoides* (van Goor) P.C. Silva along the Mid-Atlantic USA coast (Chapman, 1998), and of *Undaria* Suringar in western Europe (Curiel *et al.*, 1998). We need to make a molecular comparison of type locality material of *A. hubbsii* before subsuming this species within *A. nipponicum*. *Antithamnion pectinatum* is a related but distinct southern hemisphere species from New Zealand.

Another Ceramiaceae species that has been widely reported from the Pacific and Atlantic Oceans, and the western Mediterranean is *A. spirographidis* (e.g., Feldmann-Mazoyer, 1941; Wollaston, 1968; Lindstrom & Gabrielson, 1989; Athanasiadis, 1996). Our phylogenetic analyses indicate that the sequences of all the Pacific and Atlantic material are identical. It is possible that a single species is distributed throughout these oceans.

Acknowledgements

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