

On the life history of *Pseudochorda nagaii* (Pseudochordaceae fam. nov.) and its transfer from the Chordariales to the Laminariales (Phaeophyta)

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The phenology and life history in culture of *Pseudochorda nagaii*, previously placed in the Chordariales, were studied. At Hanasaki, Hokkaido, Japan, the species is an annual, appearing in June, maturing in November–December, and disappearing the following July–August. In culture this species alternates between a microscopic filamentous oogamous gametophyte and a macroscopic parenchymatous sporophyte. The presence of oogamous reproduction and the parenchymatous construction of the sporophyte provide evidence that the species belongs to the Laminariales and not the Chordariales. However, *P. nagaii* differs from other Laminariales as follows: in the early developmental stage the apical segment of the sporophyte is asymmetric and lacks a phaeophycean hair; the paraphyses of the sporophyte are multicellular; and the gametophyte is monoecious and produces distinctive plurilocular antheridia. Consequently, the Pseudochordaceae fam. nov. is proposed to accommodate *Pseudochorda* within the Laminariales. Sporophytes develop and mature at temperatures of 10°C or less. Gametophytes mature in 5°C short-day conditions, which corresponds to winter in Hokkaido.

INTRODUCTION

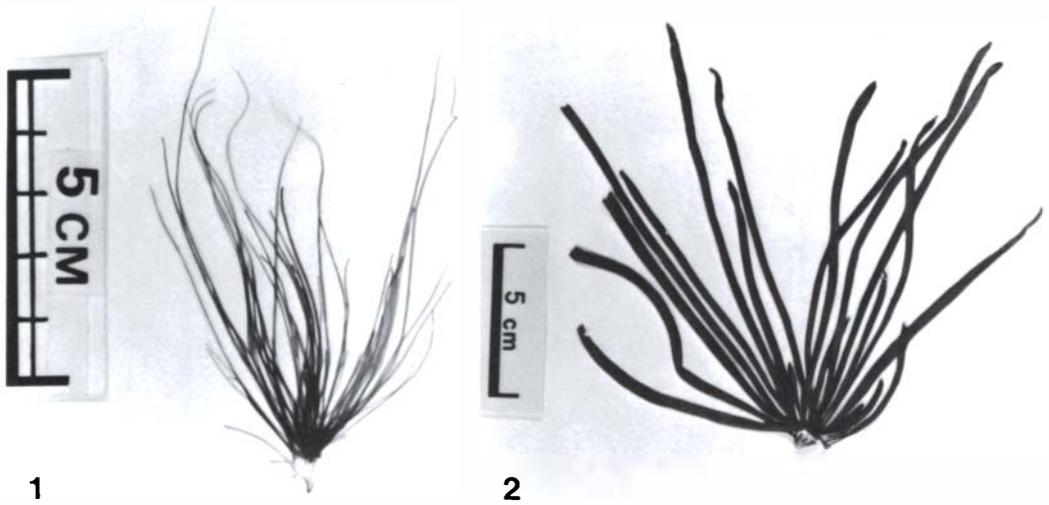
Pseudochorda nagaii (Tokida) Inagaki, the only species of the genus, has been placed in the Chordariaceae (Chordariales) since the original description by Tokida (1938) as *Chordaria nagaii*. Hori (1972), however, questioned its systematic position in the Chordariales, because the species lacks pyrenoids while most members of the order have pyrenoids. The species is reported from the Aleutian Islands (at Attu Island collected by Nobuyuki Masuda, a member of Aleutian Research Expedition of Hokkaido University during July–August 1975), Southern Kuriles [at Shikotan Island (Kawabata 1936; Nagai 1954)], Southern Saghalien (Tokida 1938, 1954), Hokkaido [at Akkeshi (Yamada & Tanaka 1944)] and Japan Sea Coast of USSR [at Zaliv Petra Velikogo (Perestenko 1980)]. Detailed morphological observations were provided by Tokida (1938) and Inagaki (1958), who noted that the plant is simple, whip-like, cylindrical, with obvious striations in surface view, caespitose, has a discoid holdfast, and is up to 60 cm [150 cm in Inagaki (1958)]. It has a medulla producing hyphae inwards and a cortex provided with uni-

seriate, multicellular paraphyses and unilocular sporangia. However, there has been no report on the phenology or life history of this species. The purpose of the present study was to provide phenological data for this species, to examine its life history in culture, and to clarify the systematic position.

MATERIALS AND METHODS

Sporophytes were collected on 27 August, 25 October, 20 December 1969; 9 April, 4 June, 17 July, 20 September 1970; 25 January, 15 April 1971; 14 May, 13 June, 1 July, 24 August, 6 December 1980 and 18 January 1981 at Hanasaki (43°17'N, 145°36'E), Hokkaido, Japan.

Cultures were established from swarmers released from unilocular sporangia on erect thalli collected on 14 May 1980. Swarmers were pipetted on to glass slides in Petri dishes and later transferred to glass vessels containing 200 ml of PESI medium (Tatewaki 1966). The medium was changed every 2–3 weeks. The sets of culture conditions used were 5°C SD (short day) (8:16), 5°C LD (long day) (16:8), 10°C SD, 10°C LD,



Figs 1, 2. *Pseudochorda nagaii* in nature at Hanasaki.
Fig. 1. Young plant attenuated toward the apex, collected on 13 June 1980.
Fig. 2. Mature plant, collected on 1 July 1980.

15°C SD, 15°C LD and 20°C LD. The isolates were pre-cultured in 10°C LD for 1 week, and then some were transferred to the other conditions except 20°C LD. Young sporophytes were cultured in all the conditions. An illuminance of 2000–3000 lux from white fluorescent tubes was used throughout.

RESULTS

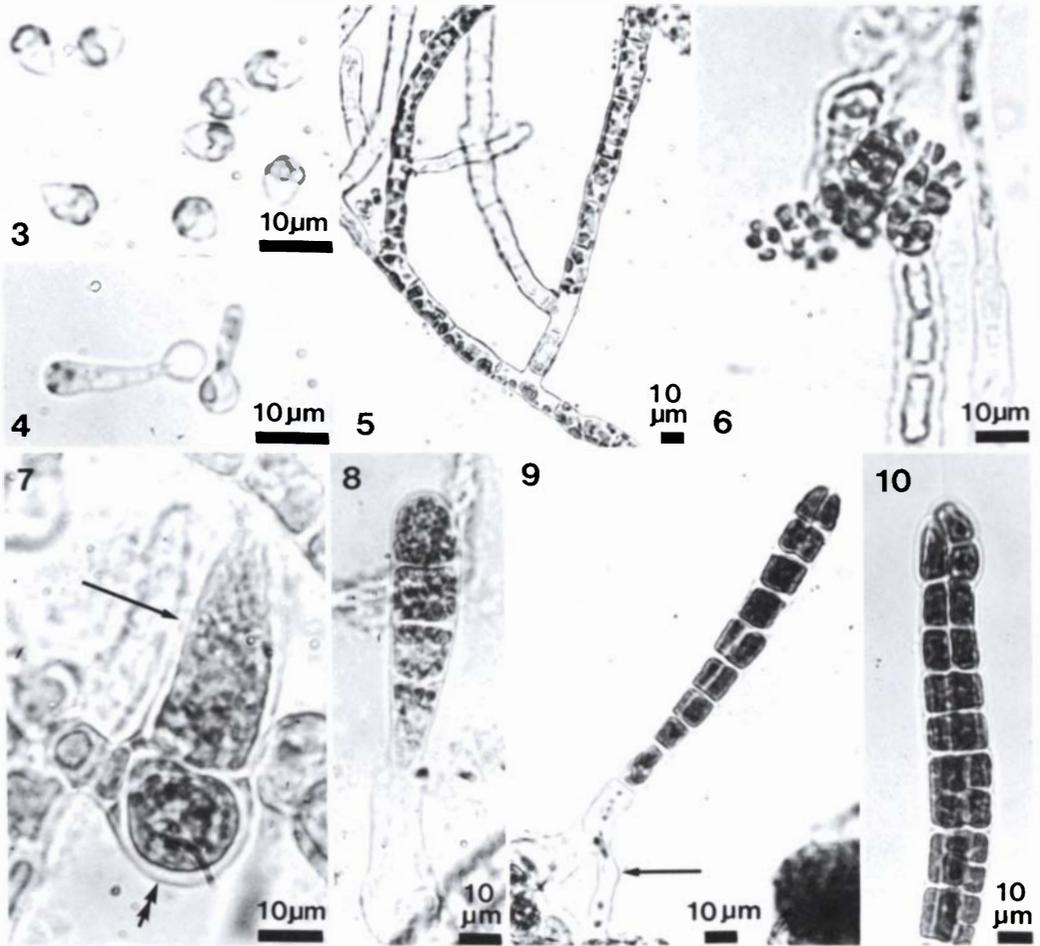
Phenology

The species grows in the lower intertidal and in the subtidal zone to 1 m deep (below Mean Low Water Level at spring tide). New macroscopic plants appear in June. Young plants (Fig. 1) are cylindrical, attenuated upward, yellowish or light brown and resemble young *Scytosiphon lomentaria* (Lyngb.) Link. They grow during summer and attain a maximum length of 30–60 cm in September, becoming darker. Unilocular sporangia form in October, and they mature in November–December. Mature, erect thalli become shorter as their upper portions are gradually lost. Their lower portions, however, last until the following July or August (Fig. 2). Regeneration from old erect thalli or discoid holdfasts were not observed.

Life history and morphology in culture

The swimmers released from unilocular sporangia are pear-shaped, 7–8 μm in length and 5–6 μm in width, and have two lateral flagella, 1–2 chloroplasts, some granules and an obvious eyespot (Fig. 3). They swim for a few minutes showing negative phototaxis, and then settle on the glass. In 1–2 days they germinate and form a germ tube (Fig. 4). Most of the cell contents of the spore migrate into the germ tube, and the original spore becomes almost empty after germination. The germlings develop into well branched uniseriate filaments (Fig. 5). Cells of the filaments contain many discoidal chloroplasts without pyrenoids. Phaeophycean hairs were not observed on the filaments. In 4–12 months, antheridia and oogonia are formed on the same filament (= monoecious gametophyte) in 5°C SD. In the other conditions tested, gametophytes did not become mature.

Antheridia are terminal or intercalary, nearly hyaline, and show a plurilocular structure formed by repeated divisions of the cells of the filament (Figs 6, 11, 12). The antheridia are lanceolate, conical or irregular in shape and 15–45 μm in length and 13–18 μm in width. Each locule contains a sperm. The sperm seems to be released from individual release pores of the locules (Fig.



Figs 3–10. *Pseudochorda nagaii*, development of gametophyte and sporophyte in culture (6, 9, 10; fixed with 5% formaldehyde and stained with 0.5% aniline blue).

Fig. 3. Zoospores.

Fig. 4. Germlings of zoospores, showing the migration of cell contents from original spore into the germination tube.

Fig. 5. Vegetative gametophytic filament of which cells containing several disc-shaped chloroplasts without pyrenoid.

Fig. 6. Plurilocular antheridium borne on gametophytic filament.

Fig. 7. Mature (single arrow) and young (double arrow) oogonia borne on gametophytic filament.

Fig. 8. Young sporophyte with transverse walls attached on the mouth of oogonium.

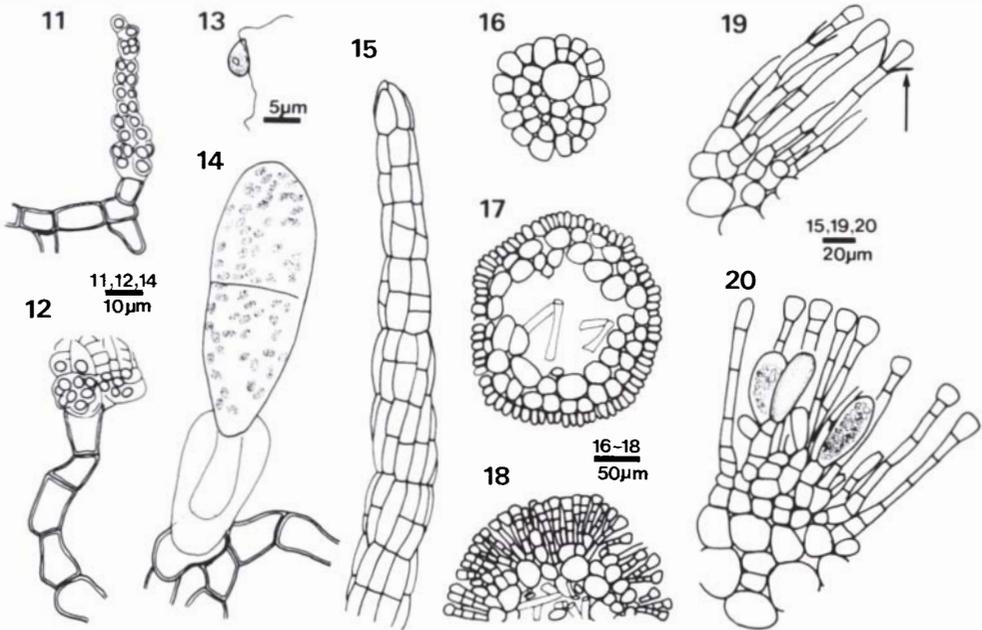
Fig. 9. Young sporophyte with several longitudinal walls, the lowermost cell of which elongates to form primary rhizoid (arrow).

Fig. 10. Upper part of young sporophyte with asymmetric apex.

12). It is nearly hyaline, pear-shape, 4–5 µm in length and 3–3.5 µm in width, has two lateral flagella, one or two chloroplasts, some granules and is devoid of an eye-spot (Fig. 13).

Oogonia are terminal or intercalary, ovoid, 33–53 µm in length and 10–25 µm in width, and contain many chloroplasts (Fig. 7). Occasionally,

two oogonia are formed side by side. Actual fertilization was not observed, but eggs extruded at the mouth of the oogonium developed into sporophytes. The first and second (and sometimes the third) cell divisions of the zygote occur in a transverse direction (Figs 8, 14). Then longitudinal divisions occur, and the thallus (em-



Figs 11-20. *Pseudochorda nagaii*, sexual reproductive organs and development of sporophyte in culture.
 Fig. 11. Lanceolate plurilocular antheridium.
 Fig. 12. Partly emptied plurilocular antheridium.
 Fig. 13. Sperm.
 Fig. 14. Two celled young sporophyte (embryo) attached on the mouth of oogonium.
 Fig. 15. Upper part of multiserial solid sporophyte showing slightly spiral arrangement of longitudinal cell rows.
 Fig. 16. Upper solid part of young sporophyte in cross section.
 Fig. 17. Sterile hollow sporophyte with inner hyphal filaments in cross section.
 Fig. 18. Upper part of adult sporophyte with multicellular paraphyses and inner hyphal filaments in cross section.
 Fig. 19. Hyaline membranous appendages (arrow) on cross walls of paraphyses.
 Fig. 20. Multicellular paraphyses and unilocular sporangia of adult plant in cross section.

bryo) becomes parenchymatous (Fig. 9). The first longitudinal walls are slightly oblique, and thus the thallus develops slightly spirally (Figs 10, 15). The lowermost cell of the young sporophyte elongates downward to form a primary rhizoid (Fig. 9). The young sporophyte elongates by further intercalary cell divisions, and then develops into a multiserial solid cylindrical thallus (Figs 15, 16). The longitudinal cell rows are still slightly spirally arranged. As the thallus grows, the spiral arrangement of the longitudinal cell rows becomes more conspicuous, and the thallus becomes hollow issuing inner hyphal filaments (Figs 17, 18, 21c, 22, 26). An obvious localized meristem could not be distinguished, but further development of the thallus occurs mainly in the middle or upper part, and the upper part of the thallus usually remains as thin solid threads (Figs

21a, b). The spiral arrangement of longitudinal cell rows are shown experimentally as the thallus in culture is easily loosened into a belt-shaped fragment along the spiral cell rows when physically stretched (Fig. 23). Well developed sporophytes grow to 20-25 cm tall and to 1.5(-5) mm in diameter (Fig. 24). Many secondary rhizoids issue from the surface of the lowermost part of the thallus (Fig. 27). Disc-shaped holdfasts as observed in the field were not formed in culture. Phaeophycean hairs were not observed on the sporophytes.

The sporophytes form paraphyses and unilocular sporangia after 3-8 months in 5°C SD, 5°C LD, 10°C SD and 10°C LD. In 15°C SD and 15°C LD, they do not grow or form reproductive organs. Most of the isolates did not survive in 20°C LD. The shedding of the outer cuticle of the thal-

lus often preceded the development of paraphyses (Fig. 25). The paraphyses are simple, composed of 4–6 cells, 48–83 μm in length (Figs 18, 19, 20, 26). Hyaline membranous appendages are often observed on the cross-walls of paraphyses (Fig. 19). The terminal cells of the paraphyses are larger than the other cells, 6–16 μm in length and 8–15 μm in width. Unilocular sporangia are usually sessile on the basal cells of the paraphyses, obovoid, 38–45 μm in length and 13–18 μm in width (Fig. 20). Phaeophyceyan hairs were not observed.

The swimmers liberated from the unilocular sporangia in culture developed in the same way as the initial swimmers from the field-collected plants.

DISCUSSION

The culture results (i.e. that *Pseudochorda nagaii* alternates between a microscopic filamentous oogamous gametophyte and a polystichous macroscopic sporophyte) provide conclusive evidence that the species belongs to the order Laminariales rather than the order Chordariales where it has been placed by previous authors (Tokida 1938, 1954; Inagaki 1958). However, *Pseudochorda nagaii* differs from previously known members of the Laminariales in some morphological features. To clarify the systematic position of the species within the order, a comparison of the features of *P. nagaii* with those of the Chordaceae [*Chorda tomentosa* Lyngbye (Maier 1984), and *Chorda filum* (Linnaeus) Lamouroux (Kanda 1938; Fritsch 1945; Henry & Cole 1982)], the Phyllariaceae (Hamel 1931–39; Tilden 1935; Henry 1984) and the other families of the order (Kanda 1938; Fritsch 1945; South & Burrows 1967; Henry & Cole 1982) is shown in Table 1. From this table, it can be seen that the plurilocular antheridia and multicellular paraphyses of *Pseudochorda nagaii* are unique within the Laminariales. Henry & Cole (1982) reported the occasional occurrence of antheridia in clusters in some members of the Laminariales and speculated that the singly borne antheridia may be regarded as a reduced plurilocular structure. From this point of view, the plurilocular antheridia of *Pseudochorda nagaii* may be regarded as the primitive type of antheridium in the order. *Pseudochorda nagaii* seems to have greater similarity with the Chordaceae than the other families in having annual cylindrical sporophytes lacking

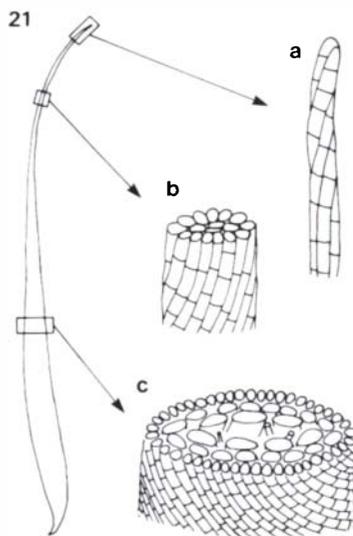
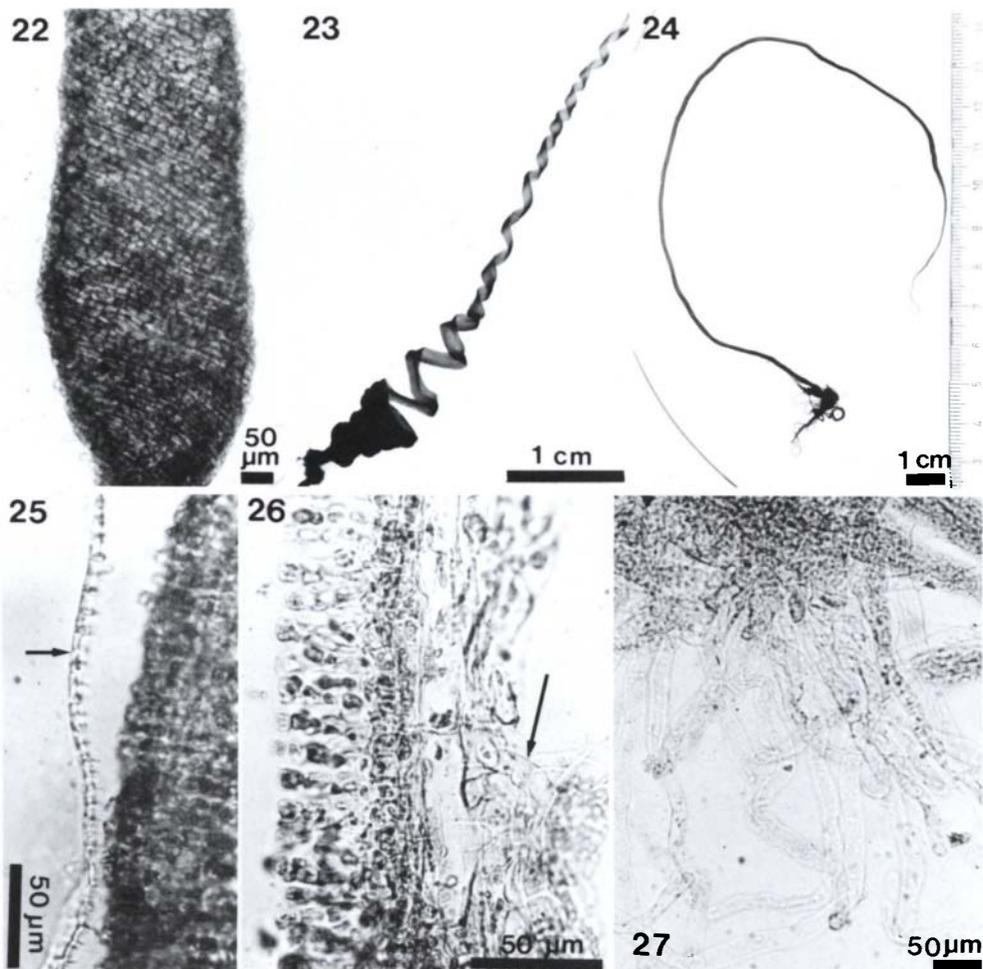


Fig. 21. A diagram showing the spiral structure of sporophyte of *Pseudochorda nagaii* in culture; a, uppermost solid portion, b, transitional solid portion, c, lower hollow portion with conspicuously spirally arranged longitudinal cell rows.

mucilaginous structures and in having eye-spots in zoospores. Within the family, it has the strongest affinity to *Chorda tomentosa* in the monoeicism of gametophyte and the lack of localized meristem in sporophyte.

Henry & Cole (1982) considered the Chordaceae to be the most primitive in the order because *Chorda filum* has eye-spots in the zoospores, trichothallic embryonic development, hairs on the surface of the adult plant, a distal intercalary meristem, and a rhizoidal holdfast. They also regarded the short posterior flagella in sperms in *Chorda filum* (Kanda 1938) as a primitive character, but Maier (1984) found that the posterior flagella in *Chorda tomentosa* have thin terminal whiplashes and are longer than the anterior ones. In addition, he considered that *Chorda tomentosa* has a more distant relationship to *Chorda filum* than hitherto considered, as it lacks a localized intercalary meristem and has monoecious gametophytes and hair-like assimilators instead of hyaline phaeophyceyan hairs. According to Henry & Cole (1982), members of the Phyllariaceae are also considered to be primitive in the order mainly because of the lack of mucilaginous organs, occurrences of apical hairs on the embryos and presence of eye-spots in zoospores of some members of them. In addition, Henry (1984) reported the occurrence of a mon-



Figs 22-27. *Pseudochorda nagaii*, morphological structure of sporophyte in culture.
Fig. 22. Middle part of sterile hollow sporophyte showing the conspicuous spiral arrangement of longitudinal cell rows of the thallus.
Fig. 23. Physically loosened sporophyte to show the spiral structure.
Fig. 24. Habit of well developed sporophyte in culture.
Fig. 25. Sloughing outer cuticle (arrow) of the thallus preceding the formation of paraphyses in cross section.
Fig. 26. Longitudinal section of adult sporophyte with multicellular paraphyses and inner hyphal filament (arrow).
Fig. 27. Secondary rhizoidal filaments issuing from surface cells of lower part of sporophyte.

oecious gametophyte in *Saccorhiza* (*Phyllaria*) *dermatodea* (De la Pyl.) J. Ag. Therefore, the phylogenetic relation between *Pseudochorda nagaii*, and the members of the Chordaceae and Phyllariaceae seems to be very important, but unfortunately, our knowledge of the Phyllariaceae is not sufficient to make detailed comparisons at present. Among characters which are regarded to be primitive, *Pseudochorda nagaii* has a monoecious gametophyte, a sporophyte

lacking mucilaginous structures and localized meristem, and zoospores with eye-spots. Additionally, the species has plurilocular antheridia and multicellular paraphyses which are unique in the Laminariales. Therefore, we regard *Pseudochorda nagaii* as one of the most primitive members of the Laminariales, and propose to establish a new family within the Laminariales to accommodate the species.

At Hanasaki, new plants appeared in spring to

Table 1. A comparison of some morphological features between *Pseudochorda nagaii*, Chordaceae (*Chorda tomentosa* and *Chorda filum*), Phyllariaceae and the other families of the Laminariales

	<i>Pseudochorda nagaii</i>	Chordaceae		Phyllariaceae	Laminariaceae, Alariaceae and Lessoniaceae
		<i>Chorda tomentosa</i>	<i>Chorda filum</i>		
Gametophyte	monoecious	monoecious	dioecious	monoecious or dioecious	dioecious
Antheridium	plurilocular structure	single celled	single celled	singled celled (?)	single celled
Sporophyte Construction	annual cylindrical (helical)	annual cylindrical	annual cylindrical (helical)	annual foliose	annual or perennial foliose
Localized meristem	-	-	+	+	+
Phaeophycean hair on embryo	-	-	+	+ or -	-
Phaeophycean hair on adult thallus	-	-	+	+	+ or -
Hair-like assimilator	-	+	-	-	-
Mucilage gland cell and/or duct	-	-	-	-	+
Paraphysis	multicellular	unicellular	unicellular	unicellular	unicellular
Mucilage cap	-	-	-	-	+ or -
Eye-spot in zoospore	+	+	+	+ (?)	-

early summer and matured in winter. Some of the mature plants remained during the following spring and disappeared in July or August. Regeneration from old plants or holdfasts was not observed, and this species is considered to be annual. In culture, the gametophytes formed oogonia and antheridia only under low temperatures (5°C) and during short days; this corresponds to winter at Hanasaki. On the other hand, the sporophytes matured in 5°C and 10°C, irrespective of daylength. From these results, the seasonal change of growth of the species seems to be controlled mainly by the maturation of the gametophyte in winter.

As for the vegetative structure of the sporophyte, the spiral arrangement of longitudinal cell rows of the thallus seems to explain the obvious striations on the surface of the thallus in nature mentioned by Tokida (1938). Another example of similar thallus spiral structure is seen in *Chorda filum* (L.) Lamour. (Greville 1830; Reinke 1892). The shedding of the outer cuticle as a normal developmental stage of the thallus in other brown algae was previously reported in *Ascophyllum nodosum* (L.) Le Jolis (Filion-Myklebust & Norton 1981). In *A. nodosum*, some of the cellular materials are sloughed off with the walls of the epidermis. In *Pseudochorda nagaii*, however, cellular materials were not observed to scatter among the shedded cuticles. Such shed-

ding of cuticles seems to be responsible for the formation of unique hyaline membranous appendages of paraphyses observed in culture as well as in nature (Tokida 1938).

Pseudochordaceae fam. nov.

Sporophyton macroscopicum, cylindricum, parenchymaticum, fistulosum, cum hyphis, cellulis medullaribus et paraphysibus multicellularibus sine pileo mucilagineo, sine pilis phaeophycearum, sine cellulis glandiferis mucilaginis. Gametophyton valde minutum, monoicum, cum antheridiis plurilocularibus ogonisque ovoideis unicellularibus.

GENUS TYPI: *Pseudochorda* Yamada et Tokida et Inagaki (Inagaki, 1958, pp. 174, 175).

Sporophyte macroscopic, cylindrical, parenchymatous, hollow, with hyphae, medullary cells and multicellular paraphyses without mucilage cap, phaeophycean hairs, and mucilage gland cells. Gametophyte minute, monoecious, with plurilocular antheridia and single-celled ovoid oogonia.

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