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A Taxonomic Study of the Seagrass Genus *Halophila* (Hydrocharitaceae) from Japan: Description of a New Species *Halophila japonica* sp. nov. and Characterization of *H. ovalis* Using Morphological and Molecular Data

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Abstract *Halophila japonica* sp. nov. is described from Japan. Although this entity has long been referred to as *H. ovalis*, data obtained from detailed morphological examination of field collections and herbarium specimens, geographical distribution records and ITS sequence analyses demonstrate that it is distinguishable from all other members of this genus and can be recognized as a new species. *H. japonica* is presently reported to occur from Ibusuki (Kagoshima Prefecture, Kyushu region, Japan) in the south, to Mutsu Bay (Aomori Prefecture, Honshu region, Japan) in the north. In order to better characterize *H. ovalis* materials from Japan, some observations on this species were also provided. As an outcome of this study, there are now four species of *Halophila* known from Japan: *H. ovalis*, *H. euphlebia*, *H. decipiens* and *H. japonica*.
Key words : *Halophila japonica* sp. nov.; ITS; Japan; morphology; taxonomy.

Introduction

The seagrass genus *Halophila* (Hydrocharitaceae) currently accomodates some 15 species assigned to the following five sections: Sect. *Halophila*, Sect. *Microhalophila*, Sect. *Spinulosae*, Sect. *Tricostatae*, and Sect. *Americanae* (den Hartog and Kuo, 2006). Of these, section *Halophila* is the largest, including some ten species and 3 subspecies which are mainly characterized as follows: Monoecious or dioecious, minute to robust plants with extremely short erect lateral shoots bearing two scales at the base and a pair of petiolate leaves at the top. Petioles are not sheathing or sheathing lopsidedly, usually longer or as long as the leaf blades. Leaf blades have ascending cross veins, smooth or serrate margins and glabrous or hairy surfaces. Female flowers are provided with 3 to 6 styles (den Hartog, 1970; Kuo and den Hartog, 2001). Members of this section are reported to be widespread in all tropical waters, extending also into subtropical and warm-temperate seas (den Hartog, 1970; Kuo and den Hartog, 2001).

In Japan, the identities and diversity of *Halophila* species are relatively little known. The most commonly recorded species is *H. ovalis* (R. Brown) J. D. Hooker (Matsumura, 1895; Makino, 1912; Miki, 1934a, 1934b; Tanaka *et al.*, 1962a, 1962b; den Hartog, 1970; Terada, 1981; Tsuda and Kamura, 1990; Toma, 1999; Kuo *et al.*, 2001; Aioi and Nakaoka, 2003; Tanada *et al.*, 2005; Kirihara *et al.*, 2005). The occurrence of a second species, *H. decipiens* Ostenfeld was only recently reported by Kuo *et al.* (1995) from Okinawa Island. According to Aioi and Nakaoka (2003), Japanese *H. ovalis* has a very wide range of geographical distribution, extending from the

Ryukyu Islands (Yaeyama) to central Honshu region (Odawa Bay in the Pacific Coast and Toyama Bay in the Japan Sea Coast), whereas *H. decipiens* is restricted to Ryukyu Islands.

Another species, *H. euphlebia* Makino has earlier been described from Japan (Shishikui in Shikoku Island) by Makino (1912) but, it was subsequently treated by Miki (1934a) as a simple morphological form of *H. ovalis*. Den Hartog (1970) in his monograph "The sea-grasses of the world" also treated *H. euphlebia* as a synonym of *H. ovalis*. Recent morphological and molecular studies by Uchimura *et al.* (2006), however, demonstrated that *H. euphlebia* clearly differs from currently established *Halophila* species. Accordingly, they proposed the reinstatement of Makino's *H. euphlebia*, thus bringing the current number of *Halophila* species to sixteen, of which three species are presently recognized in Japan: *H. ovalis*, *H. decipiens* and *H. euphlebia*.

An ongoing taxonomic re-evaluation of Japanese members of this genus using morphological and molecular techniques, however, led to strongly suspect that some unreported or unidentified species may still be present in Japanese waters (Uchimura *et al.*, 2005, 2006).

In this paper, some *Halophila* plants from Honshu, Shikoku and Kyushu regions which have been misidentified as *H. ovalis* (Makino, 1912; Miki, 1934a, 1934b; Mukai *et al.*, 1980; Tanada *et al.*, 2005; Kirihara *et al.*, 2005) are described as a new species (*Halophila japonica* sp. nov.) based on detailed observations of vegetative and reproductive features, distributional characteristics, and sequences analyses of the nuclear ribosomal internal transcribed spacer (ITS) region including the 5.8S gene. In an effort to facilitate species identification, morphological characteristics and geographic distribution of typical *H. ovalis* materials from Japan were also re-examined and documented here.

Materials and Methods

Morphological observations

Specimens were collected either by SCUBA diving or snorkeling from the sites listed in Table 1. After each collection, some portions of samples were desiccated in silica gel for later DNA extraction, the remaining transported to the laboratory for measurements and detailed morphological examinations. Photographs were taken using an OLYMPUS DP 50 (OLYMPUS Co., Tokyo, Japan) digital camera mounted on an OLYMPUS SZX 12 (OLYMPUS Co., Tokyo, Japan) stereo microscope. The specimens were later processed as dried herbarium voucher specimens or stored at −20°C. Those representative herbarium specimens are currently deposited at the herbarium of the National Science Museum, Tokyo (TNS) located at Tsukuba city, Japan.

Additional voucher dried specimens which are currently housed in established herbaria including TNS, the herbarium of the University Museum, University of Tokyo (TI) and Makino herbarium (MAK), Tokyo Metropolitan University were also examined.

DNA extraction, gene amplification, sequencing and phylogenetic analysis

DNA samples were extracted from fourteen specimens listed in Table 1, with their collection information and code. Primers and protocols for DNA extraction, ITS amplification and automated sequencing are as previously published (Uchimura *et al.*, 2006).

Phylogenetic analyses were performed using the Maximum Parsimony (MP) and Maximum Likelihood (ML) algorithms available in the computer program PAUP V. 4.0 b10 (Swofford, 2002). Identical sequences within each species were excluded from the alignment. Additional sequences were obtained from the NCBI/GenBank database (Table 2) and included in the alignment. Gaps were considered as missing data. Outgroup species consisted of *Halophila beccarii* Ascherson (Genbank accession number AF366441) and *H. engelmanni* Ascherson (Genbank accession number AF366404). They were chosen following Waycott *et al.* (2002). The alignment is available from the third author upon request.

Table 1. List of *Halophila japonica* specimens used in molecular analyses.

Collection information and sample code	TNS herbarium voucher number	Reproductive state
1. Suou-Ooshima, Yamaguchi Prefecture, 28 Jul. 2004, [HA35]	TNS 753649	Staminate
2. Suou-Ooshima, Yamaguchi Prefecture, 28 Jul. 2004, [HA36]	TNS 753650	Staminate
3. Mugi-Ooshima (33°38′10″N/134°29′13″E), Tokushima Prefecture, 3 Jun. 2005, [HJ3]	TNS 753651	Sterile
4. Odawa Bay (35°13′16.2″N/139°37′17.4″E), Kanagawa Prefecture, 18 Nov. 2004, [HA54]	TNS 753655	Sterile
5. Odawa Bay (35°13′16.2″N/139°37′17.4″E), Kanagawa Prefecture, 7 Jun. 2005, [HA24]	TNS 753656	Pistillate
6. Odawa Bay (35°13′16.2″N/139°37′17.4″E), Kanagawa Prefecture, 7 Jun. 2005, [HA25]	TNS 753658	Staminate
7. Urumi (36°01′48.5″N/133°01′29.0″E) Chibu Island, Shimane Prefecture, 1 Sept. 2005, [HJ10]	TNS 753668	With fruits
8. Mukoujima, Naoshima (34°27′55″N/133°59′54″E), Kagawa Prefecture, 24 Aug. 2005, [HJ11]	TNS 753670	Staminate
9. Oohama, Takuma (34°13′33″N/133°36′35″E), Kagawa Prefecture, 4 Aug. 2005, [HJ12]	TNS 753671	Pistillate
10. Mihokogaura Beach (32°25′19.0″N/131°40′30″E), Miyazaki Prefecture, 26 Sept. 2005, [HJ13]	TNS 753672	Sterile
11. Koshiki Island (31°51′04″N/129°55′02″E), Kagoshima Prefecture, 28 Sept. 2005, [HJ14]	TNS 753673	Sterile
12. Sasebo (33°10′50.2″N/129°38′46.8″E), Nagasaki Prefecture, 30 Sept. 2005, [HJ15]	TNS 753674	Sterile
13. Hasama (34°58′30.6″N/139°46′58.7″E), Tateyama, Chiba Prefecture, 18 Oct. 2005, [HJ16]	TNS 753675	Sterile
14. Mutsu Bay (41°00′35.5″N/140°40′01.4″E), Aomori Prefecture, 31 Oct. 2005, [HJ17]	TNS 753676	Sterile

In the MP analysis, all characters and character changes were treated as unordered and equally weighted. Heuristic search was performed with TBR and MULTREES activated and consisted of 1000 replicates of random sequence addition. Support for the nodes of the MP tree was determined by calculating bootstrap values (Felsenstein, 1985) based on 2000 replications of full heuristic searches.

ML analysis was performed under the Jukes-Cantor model (Jukes and Cantor, 1969) following Takahashi and Nei (2000) who mentioned that in the presence of a large number of short sequences it is better using a simple model of nucleotide substitution. The data set was analyzed by a heuristic search with the following options: starting tree=NJ tree, branch swapping algorithm=TBR. ML bootstrap analysis was based on 100 replicates of the data set (Felsenstein, 1985).

Results

Halophila japonica Uchimura & Faye, sp. nov.
Figs. 1–12

Diagnosis. *Repentes herbulae submarinae, annuae vel perennes; rhizoma 0.6–1.5 mm diametro, albida vel hyalina, ad nodos radicantes, foliates; squamis ad nodos binis, usque ad 5 mm longis; internodia 1–4 cm longa; folia bina petiolata, glabra, margine integra, oblonga vel obovata, ad apice obtusa vel rotundata, ad basim angustata, 0.8–30 mm longa, 3–12 mm lata, venae transversae utroque latere 6–12; nervus intramarginalis 0.2–1 mm margine remotus, petioli usque ad 3 cm longi. Flores dioici, solitarii. Flores masculini: bracteae 2, concavae, ad apice acutus, 6–8 mm longae; tepalae 3, oblongae, 2–4 mm longae; antherae 3, oblongae; pollen irregulare et confervoideum. Flores feminei: bracteae 2, hyalinae, imbricatae, 5–7 mm longae, ovarium sessile vel breve stipitatum, 1–2 mm longum,*

Table 2. *Halophila* species for which ITS sequences were obtained from GenBank and included in the molecular analyses.

Taxon	GenBank number
Halophila beccarii Ascherson [Vietnam]	AF366441
Halophila euphlebia Makino [Japan]	AB243957
Halophila euphlebia Makino [Japan]	AB243962
Halophila euphlebia Makino [Japan]	AB243966
Halophila engelmannii Ascherson [Florida]	AF366404
Halophila spinulosa (R. Brown) Ascherson [Malaysia]	AF366440
Halophila spinulosa (R. Brown) Ascherson [Australia]	AF366439
Halophila tricostata Greenway [Australia]	AF366438
Halophila stipulacea (Forsskal) Ascherson [Italy]	AF366436
Halophila decipiens Ostenfeld [Japan]	AB243977
Halophila decipiens Ostenfeld [Japan]	AB243983
Halophila decipiens Ostenfeld [Japan]	AB243984
Halophila decipiens Ostenfeld [Australia]	AF366411
Halophila decipiens Ostenfeld [Malaysia]	AF366412
Halophila decipiens Ostenfeld [Florida]	AF366407
Halophila ovalis (R. Brown) Hook. f. [Australia]	AF366429
Halophila ovalis (R. Brown) Hook. f. [Vietnam]	AF366437
Halophila ovalis (R. Brown) Hook. f. [Malaysia]	AF366420
Halophila ovalis (R. Brown) Hook. f. [Philippines]	AF366416
Halophila ovalis (R. Brown) Hook. f. [Philippines]	AF366417
Halophila ovalis (R. Brown) J. D. Hooker [Japan]	AB243970
Halophila ovalis (R. Brown) J. D. Hooker [Japan]	AB243975
Halophila ovalis (R. Brown) J. D. Hooker [Japan]	AB243976
Halophila hawaiiana Doty et Stone [Hawaii]	AF366426
Halophila johnsonii Eiseman [Florida]	AF366425
Halophila minor (Zollinger) den Hartog [Guam]	AF366405
Halophila minor (Zollinger) den Hartog [Philippines]	AF366406
Halophila australis Doty et Stone [Australia]	AF366414

0.5–1 mm diametro, hypanthium 1–6 mm longum et in 3 filiformis stylis 6–20 mm longis divisis. Fructus globosus 2.5–3 mm diametro; 5–13 globosa semina cum reticulata testa, 0.4–1 mm diametro.

Description. Prostrate marine plant; annual or perennial; rhizome irregularly branched, creeping, whitish or transparent, 0.6–1.5 mm thick; roots borne singly underneath each node; scales two at nodes, up to 5 mm long; internodes 1–4 cm long; leaves petiolate, in pairs at each node, glabrous, margin entire, oblong to obovate in shape, obtuse or rounded at apex, attenuate at base, 0.8–30 mm long, 3–12 mm wide, cross veins 6–12 at each side of a midrib; distance between leaf margin and the intramarginal vein 0.2–1 mm wide; petioles up to 3 cm long. Flowers are solitary and dioecious. Male flower: enveloping bracts 2, concave, acute at apex; tepals 3, oblong, 6–8 mm long; anthers 3, oblong; pollen grains irregular in shape and in loose chains. Female flower: enveloping bracts 2, transparent, imbricate, 5–7 mm long; ovary sessile or shortly stipitate, 1–2 mm long, 0.5–1 mm in diameter; hypanthium 1–6 mm long, subdivided at the top into 3 filiform styles, 6–20 mm long. Fruits globose, 2.5–3 mm in diameter; seeds coated by a reticulate testa, 5–13 in number, 0.4–1 mm in diameter.

Etymology. The specific epithet "*japonica*" refers to the provenance of the new species.

Japanese name. Yamato-Umihirumo.

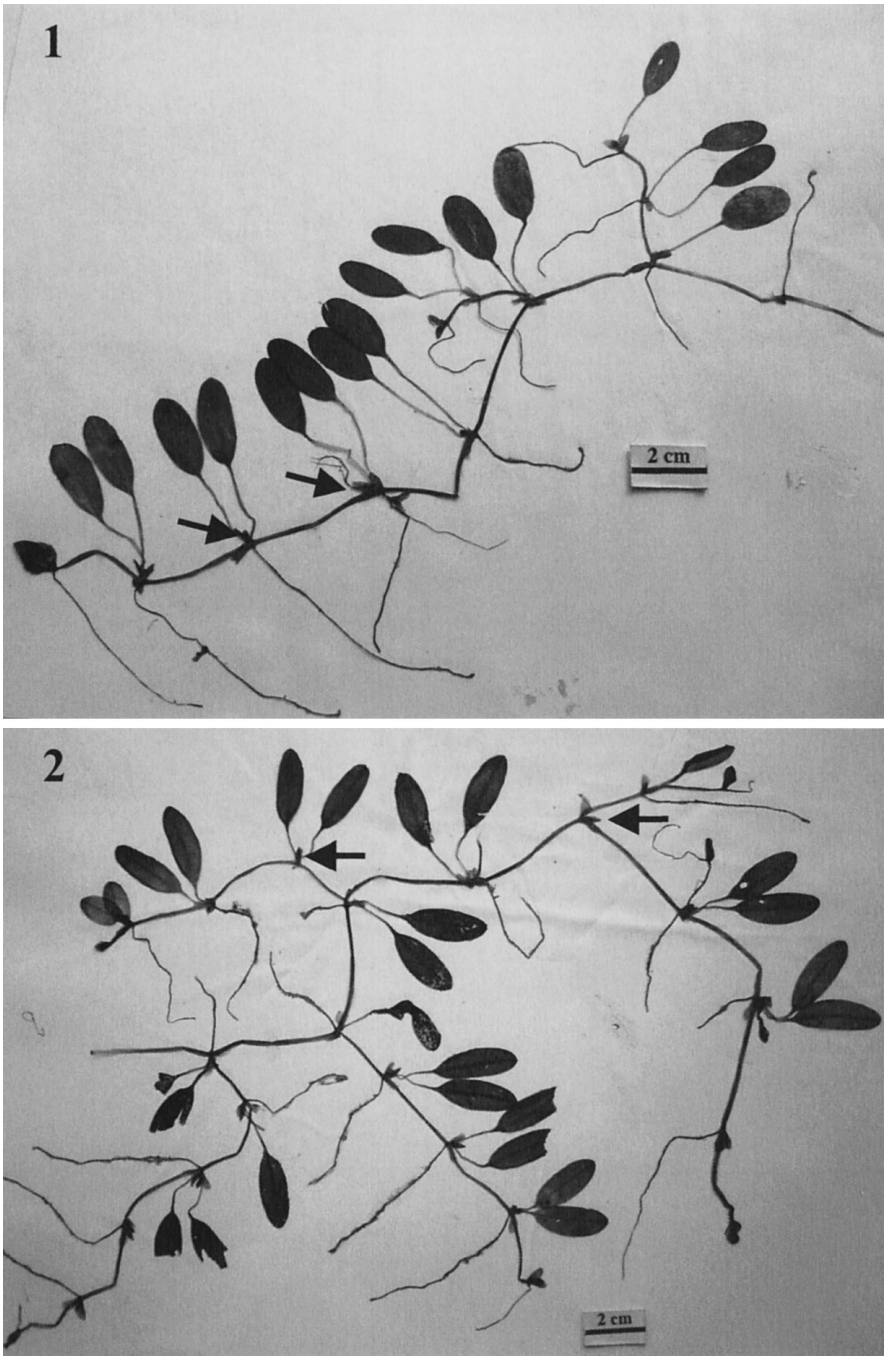
Type locality. Odawa Bay (35°13′16″N/139°37′17″E), Yokosuka City, Kanagawa Prefecture, Japan.

Holotype. TNS 753656 (Fig. 1), pistillate specimen from Odawa Bay, Yokosuka City, Japan, collected on 7 June 2005 by M. Uchimura.

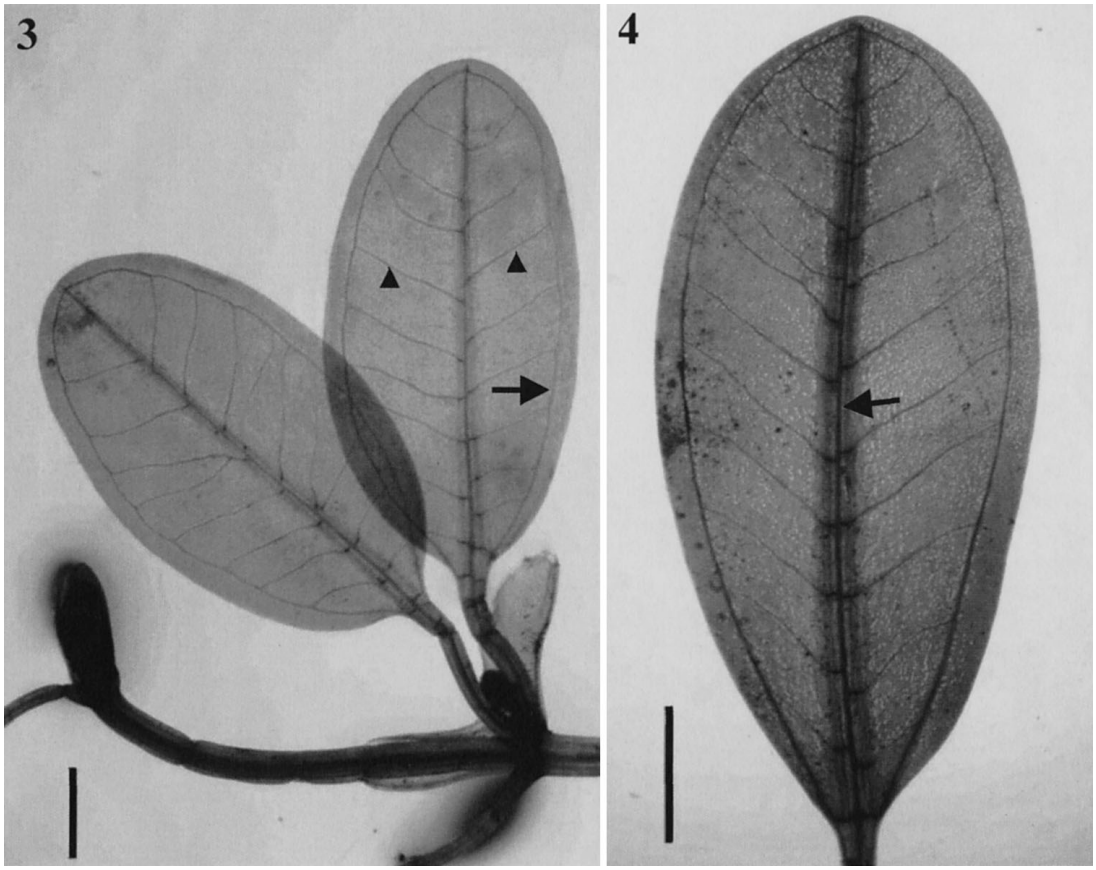
Isotypes. TNS 753657 (pistillate), TNS 753658 (staminate).

Distribution. Honshu, Shikoku and Kyushu Islands, Japan (Fig. 26). Owing to the insufficient knowledge of the global taxonomic diversity of this genus, however, we are unable to determine whether this new species is endemic to Japan or more widely distributed.

Material examined. 1) Matsugasaki, Oki Island, Shimane Prefecture (23.vii.2004, *leg.* Masayuki Uchimura, sterile, TNS 753647); 2) Takugi, Oki Island, Shimane Prefecture (23.vii.2004, *leg.* Masayuki Uchimura, sterile, TNS 753648); 3) Suou-Ooshima, Yamaguchi Prefecture (28.vii.2004, *leg.* Tsutomu Miyazaki, sterile, TNS 753649, 753650); 4) Mugi-Ooshima, Tokushima Prefecture (3.vi.2005, *leg.* Shogo

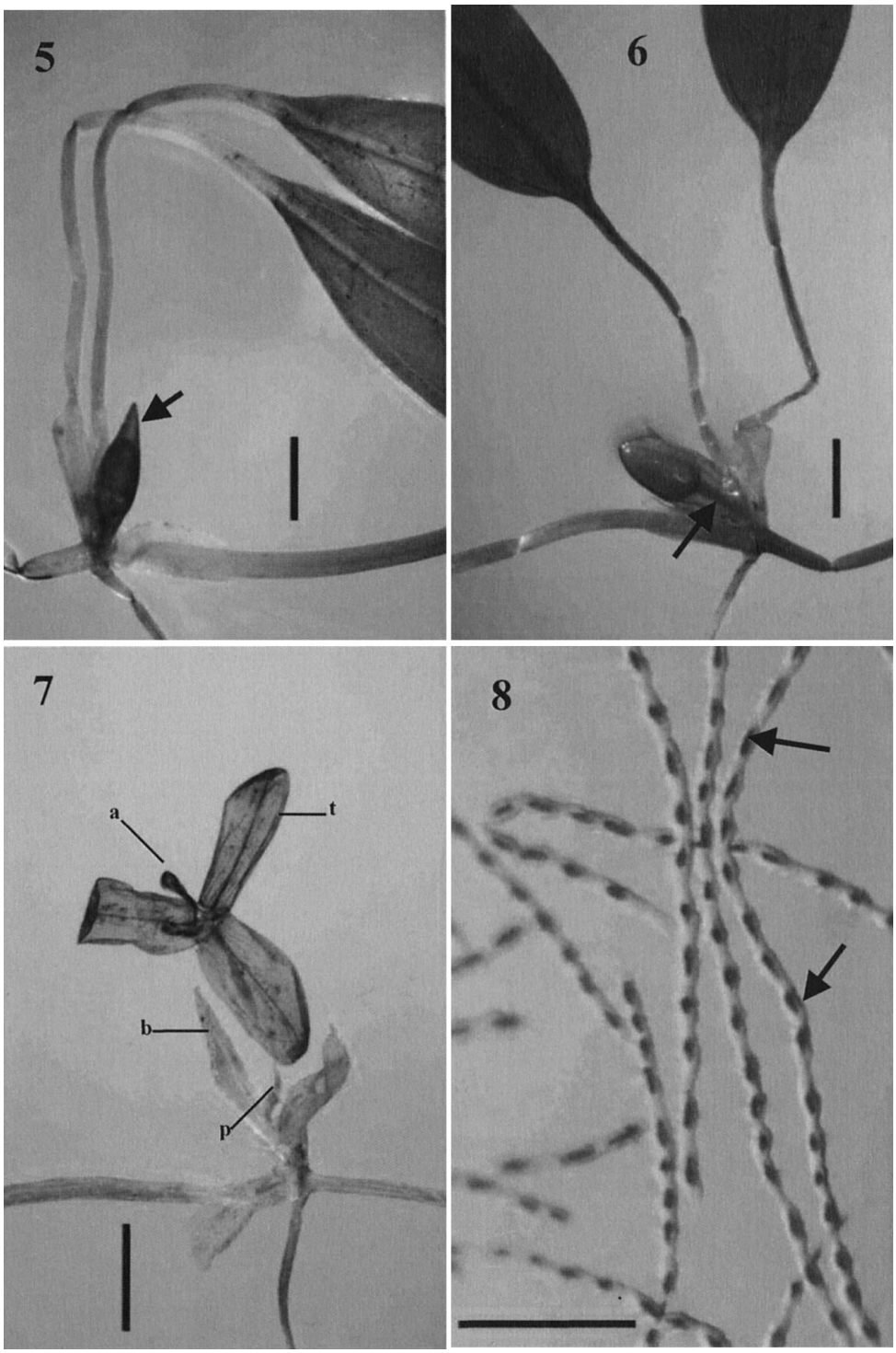


Figs. 1–2. Voucher herbarium specimens of *Halophila japonica*. (1) Holotype specimen (TNS 753656) with pistillate flowers (arrows) (Scale bar=2 cm). (2) Male plant (TNS 753658) with sessile flowers (arrows) (Scale bar=2 cm).

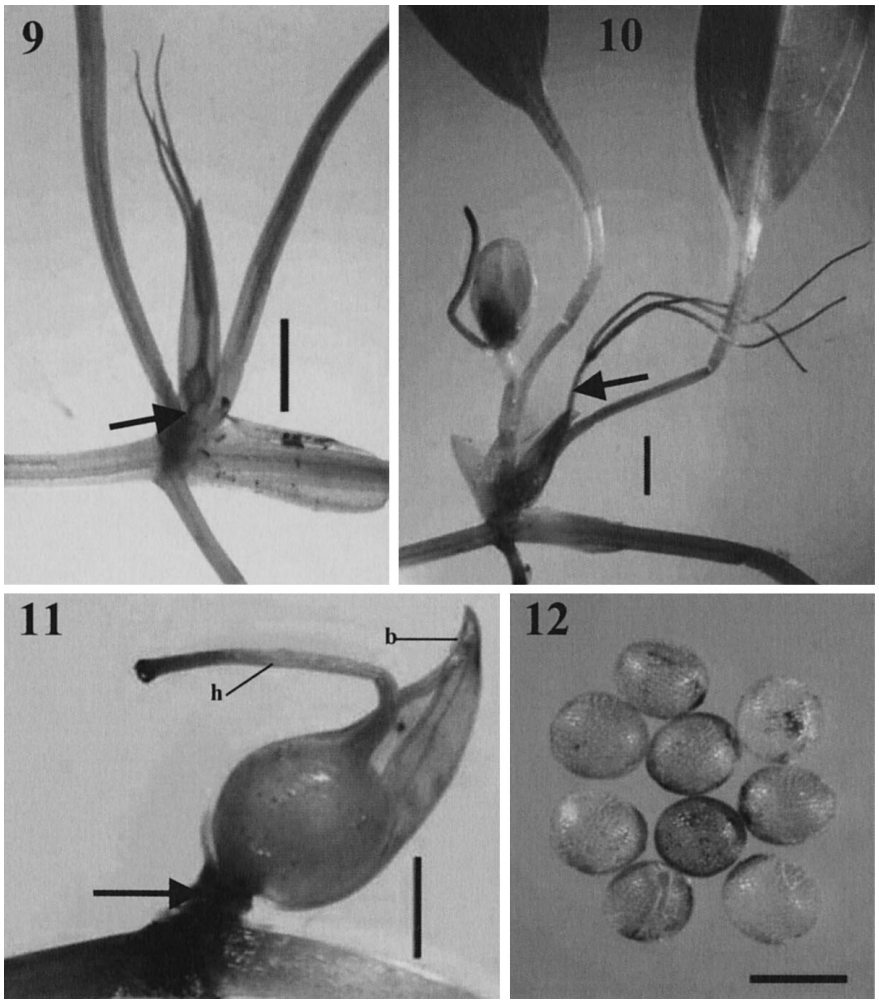


Figs. 3–4. Leaf structures of *Halophila japonica*. (3) Close-up view of a pair of young leaves showing the intramarginal vein (arrow) and cross veins (arrowheads) (Scale bar＝2 mm). (4) Close-up view of a mature leaf showing the central vein (arrow) (Scale bar＝5 mm).

Arai, sterile, TNS 753651, 753652); 5) Shishikui, Tokushima Prefecture (4.vi.2005, *leg.* Shogo Arai, sterile, TNS 753653, 753654); 6) Odawa Bay, Kanagawa Prefecture (18.xi.2004, *leg.* Masayuki Uchimura, sterile, TNS 753655); 7) Odawa Bay, Kanagawa Prefecture (7.vi.2005, *leg.* Masayuki Uchimura, pistillate, TNS 753656, 753657, staminate, TNS 753658); 8) Odawa Bay, Kanagawa Prefecture (8.vii.2005, *leg.* Masayuki Uchimura, with fruits, TNS 753659); 9) Odawa Bay, Kanagawa Prefecture (5.viii.2005, *leg.* Masayuki Uchimura, pistillate, TNS 753660, staminate, TNS 753661); 10) Odawa Bay, Kanagawa Prefecture (12.ix.2005, *leg.* Masayuki Uchimura, sterile, TNS 753662); 11) Odawa Bay, Kanagawa Prefecture (18.x.2005, *leg.* Masayuki Uchimura, sterile, TNS 753663); 12) Kirishima, Sukumo, Kochi Prefecture (8.vii.2005, *leg.* Shogo Arai, staminate, TNS 753664, 753665); 13) Nanao Bay, Ishikawa Prefecture (29.vii.2005, *leg.* Shogo Arai, pistillate, TNS 753666, staminate, TNS 753667); 14) Urumi Port, Chibu Island, Shimane Prefecture (1.ix.2005, *leg.* Masayuki Uchimura, with fruits, TNS 753668); 15) Izanaki, Nishinoshima Island, Shimane Prefecture (2.ix.2005, *leg.* Masayuki Uchimura, sterile, TNS 753669); 16) Mukoujima, Naoshima, Kagawa Prefecture (24.viii.2005, *leg.* Munehiro Fujiwara, staminate TNS 753670); 17) Oohama, Takuma, Kagawa Prefecture (4.viii.2005, *leg.* Munehiro Fujiwara, pistillate, TNS 753671); 18) Mihokogaura beach, Miyazaki Prefecture



Figs. 5–8. Male reproductive structures of *Halophila japonica*. (5) Young male flower (arrow) developing at the base of a pair of leaves. (Scale bar=3 mm). (6) Developing male flower with elongating pedicel (arrow) pushing the floral bud out of the spathal bracts (Scale bar=3 mm). (7) Opened male flower showing remnants of anthers (a), three tepals (t), one pedicel (p) and two bracts (b) (Scale bar=3 mm). (8) Pollen grains (arrows) organized into moniliform chains (Scale bar=0.5 mm).



Figs. 9–12. Female reproductive structures of *Halophila japonica*. (9) Young female flower with a short stipe (arrow) (Scale bar=2 mm). (10) Female flower with a long hypanthium (arrow) extended by three styles (Scale bar=3 mm). (11) Mature fruit with a short stipe (arrow), a persistant hypanthium (h) and one of the two enveloping spathal bracts (b) (Scale bar=2 mm). (12) Seeds provided with enveloping coat (Scale bar=1 mm).

(26.ix.2005, *leg.* Masayuki Uchimura, sterile, TNS 753672); 19) Koshiki Island, Kagoshima Prefecture (28.ix.2005, *leg.* Masayuki Uchimura, sterile, TNS 753673); 20) Sasebo, Nagasaki Prefecture (30.ix.2005, *leg.* Masayuki Uchimura, sterile TNS 753674); 21) Hasama, Tateyama, Chiba Prefecture (18.x.2005, *leg.* Masayuki Uchimura, sterile, TNS 753675); 22) Mutsu Bay, Aomori Prefecture (31.x.2005, *leg.* Masayuki Uchimura, sterile, TNS 753676); 23) Nabeta Bay, Shimoda, Shizuoka Prefecture (4.vii.1969, *leg.* Mitsuo Chihara, TNS 37715, identified as *H. ovalis* (R. Br.) Hooker); 24) Naruto city, Tokushima Prefecture (11.ix.1965, *leg.* Abe Kinichi, TNS 162594, 174931, identified as *H. ovalis*); 25) Boshu-Funakata, Chiba Prefecture (29.iii.1952, *leg.* Asano Tadao, TNS 97533, identified as *H. ovalis*); 26) Misaki Sagami, Kanagawa Prefecture (15.viii.1958, *leg.* Kazuko Arai, TNS 137635, identified as *H. euphlebia* Makino); 27) Tamasu-Gun, Uchiura town, Ishikawa Prefecture (27.viii.1962, *leg.* Satomi Nobuno,

TNS 152538, identified as *H. ovalis*); 28) Iki (15.viii.1910, *leg.* Zentaro Tashiro, TI 19766, identified as H. ovata); 29) Hizen, Kitsutsu (28.x. 1906, *leg.* Zentaro Tashiro, TI 30181); 30) Tanabe, Kii Peninsula [xi.1924, *leg.* Nakajima, TI (without name and number)]; 31) Tanabe, Kii Peninsula, Wakayama Prefecture [(month unknown) 1924, MAK 226960, identified as *H. ovalis*]; 32) Ibusuki, Kagoshima Prefecture (17.vii.1965, MAK 89739, identified as *H. ovalis*); 33) Takamatsu, Kagawa Prefecture [(date unknown), MAK 226964, identified as *H. ovalis*]; 34) Matsuyama, Ehime Prefecture (ix.1900, MAK 196341, identified as *H. ovalis*); 35) Maizuru Bay, Kyoto [(date unknown), MAK 296961, identified as *H. ovalis*]; 36) Takehara, Hiroshima Prefecture [(month unknown) 1911, MAK 296963, identified as *H. ovalis*].

Habitat and seasonality. *Halophila japonica* plants generally grow on soft and muddy or sandy sediments, extending from the lowest tide levels of calm shores down to 15 m on open coasts. Sometimes they form isolated small patches or are associated with other seagrasses such as some *Zostera* species or *H. euphlebia*. At Odawa Bay (Yokosuka City, Kanagawa Prefecture), plants are suspected to be present throughout the year as they were regularly (monthly) collected from June 2005 to May 2006. Flowers and/or fruits were observed in plants from Suou-Ooshima (Yamaguchi Prefecture), Odawa Bay (Kanagawa Prefecture), Tsukumo (Kochi Prefecture), Nanao Bay (Ishikawa Prefecture), Chibu Island (Shimane Prefecture), Naoshima (Kagawa Prefecture) and Takuma (Kagawa Prefecture) that were collected between June and September (Table 1).

Vegetative morphology. Plants are somewhat delicate, consisting of slender, creeping, intertwined rhizomes, 0.6–1.5 mm in diameter, irregularly segmented into internodes, 1–4 cm long, producing at each node a pair of petiolated leaves above and a single root beneath (Figs. 1, 2). Petioles are slender, terete to flattened, up to 3 cm long, covered at base by a transparent scale (Fig. 3), 3–5 mm long. Another scale envelops the rhizome (Fig. 3). Leaf blades are smooth along margins, glabrous, translucent green to bright green in color, oblong to obovate in shape, obtuse or rounded at apex, attenuate at base (Figs. 3, 4), 8–30 mm long, 3–12 mm wide, with 6–12 cross veins at each side of a central vein. Cross veins are alternate to sub-opposite along the central vein and rarely branched. The space between two consecutive cross veins is somewhat wide and varies between 1 and 4 mm. That between the intramarginal vein and blade margin is also relatively wide, and ranges between 0.2 and 1 mm. The intramarginal vein and central vein join at the apical portion of the leaves (Figs. 3, 4).

Reproductive morphology. Male and female flowers are borne in separate plants (Figs. 1, 2). Male flowers arise from the base of two leaves and are sessile to sub-sessile at first (Fig. 5), becoming pedicellate later (Figs. 6, 7). They are composed of two transparent and imbricate spathal bracts, 6–8 mm long, three elliptic tepals, 2–4 mm long and three anther lobes containing pollen grains which are arranged into moniliform chains (Fig. 8).

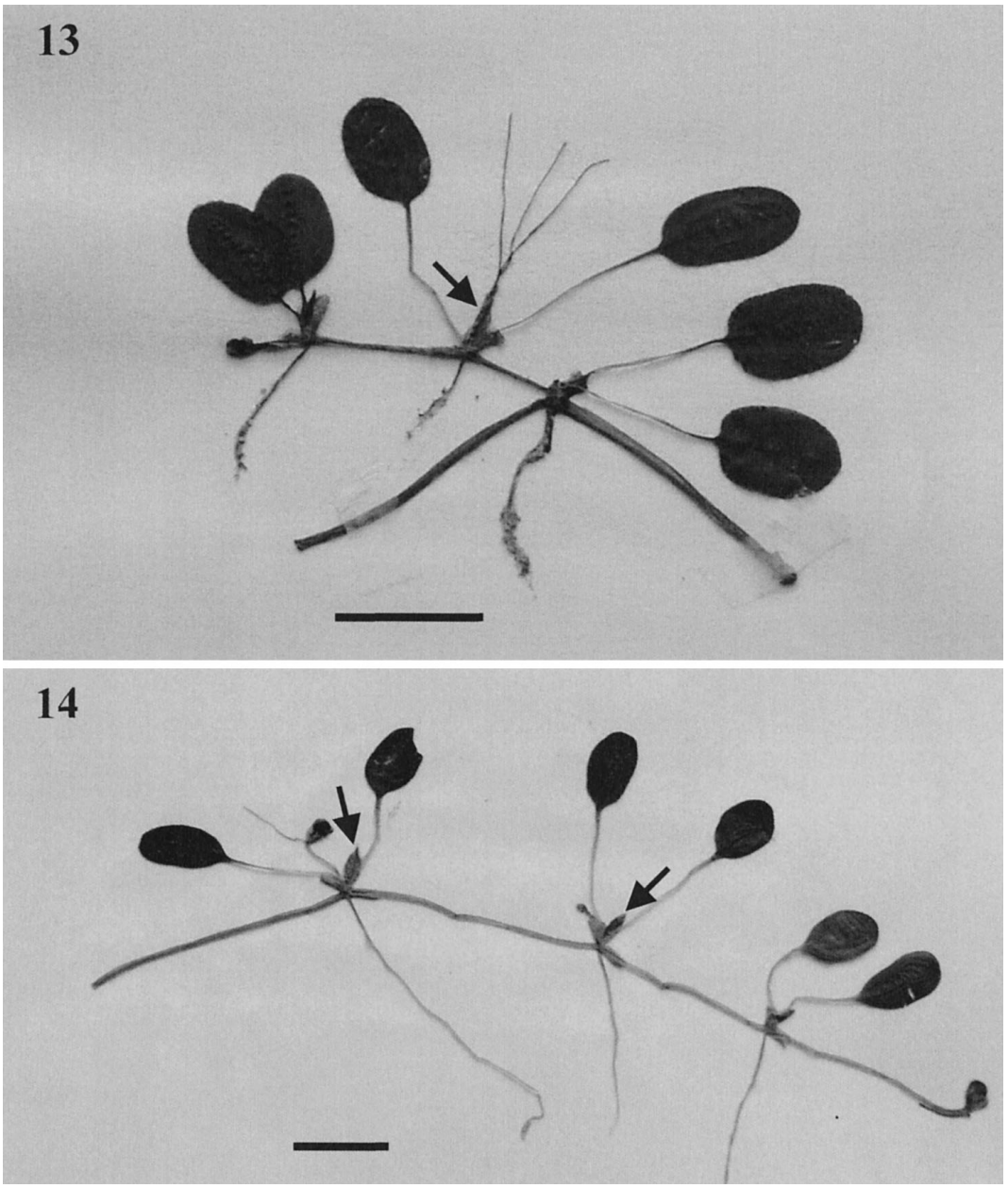
Female flowers also develop at the axil of a leaf pair. They are sessile, or shortly stipitate (Fig. 9), and consist of two spathal bracts, 5–7 mm long, enclosing an ovary that is 1–2 mm long, 0.5–1 mm in diameter (Figs. 9, 10). The ovary bears a 1–6 mm long hypanthium which is further extended by 3 (Figs 9, 10), unequal styles, 6–20 mm long. Mature fruits (Fig. 11) are ovoid to spherical, light-green to yellow-white in color, 2.5–3 mm in diameter, containing 5–13 globose seeds (Fig. 12). Seeds are whitish to yellow-brown in color, shortly beaked, 0.4–1 mm in diameter and may be visible through the thin pericarp of some mature fruits.

Halophila ovalis (R. Brown) J. D. Hooker.
Figs. 13–25.

Basionym. *Caulinia ovalis* R. Brown 1810.

Japanese name. Umihirumo.

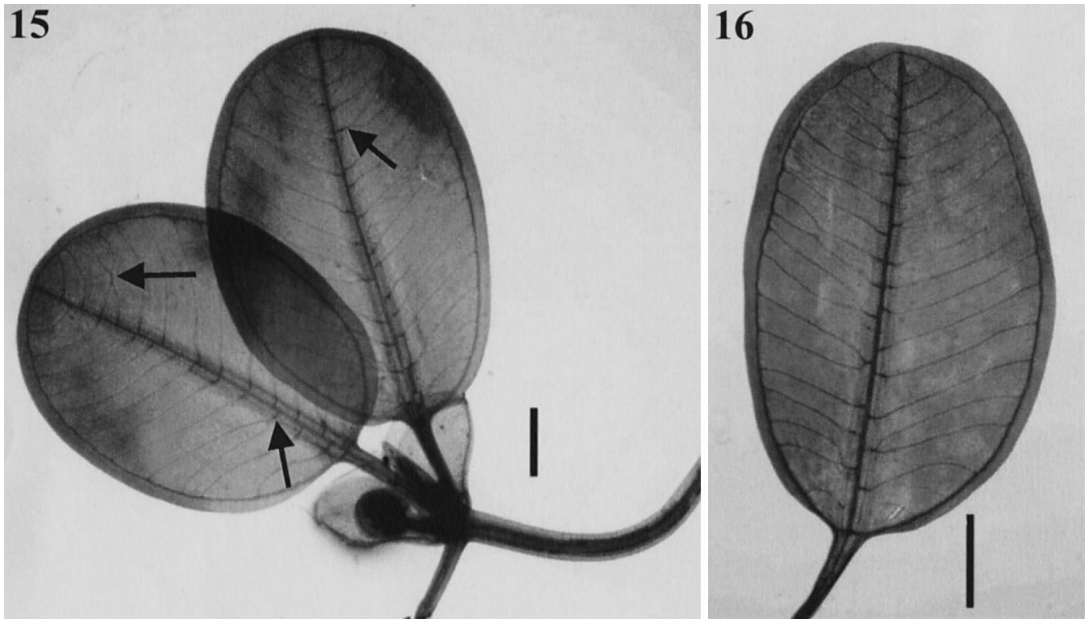
Type locality. Queensland, Australia (exact locality unknown) (*R. Brown* 5816); in BM (Womersley, 1984).



Figs. 13–14. Voucher herbarium specimens of *Halophila ovalis*. (13) Female plant (TNS 753656) with a pistillate flower (arrow) (Scale bar=2 cm). (14) Male plant (TNS 753658) with foral buds (arrows) (Scale bar=2 cm).

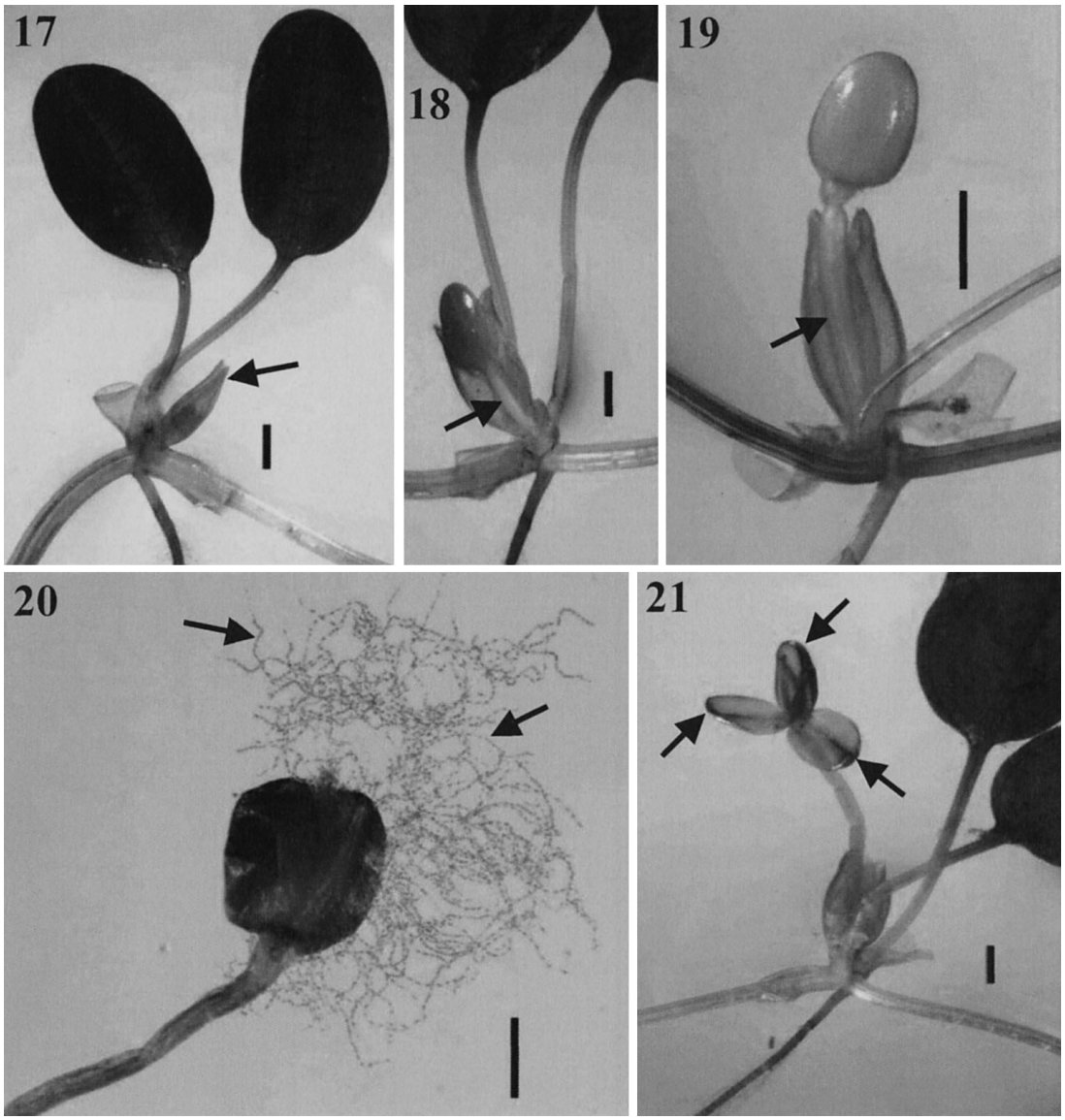
Distribution. Tropics, Indo-Pacific region, temperate Australia and Africa (Waycott *et al.*, 2002). In Japan, the species appeared to be widespread in the Ryukyu Islands (southern Japan), and does not seem to reach farther north than Amami-Ooshima Island (Fig. 26).

Material examined. 1) Nakagusuku Bay, Okinawa Prefecture (14.vi.2004, 26°18′67.1″N/127°50′31.7″E, *leg.* Masayuki Uchimura, pistillate and staminate, TNS 752690, 752691,



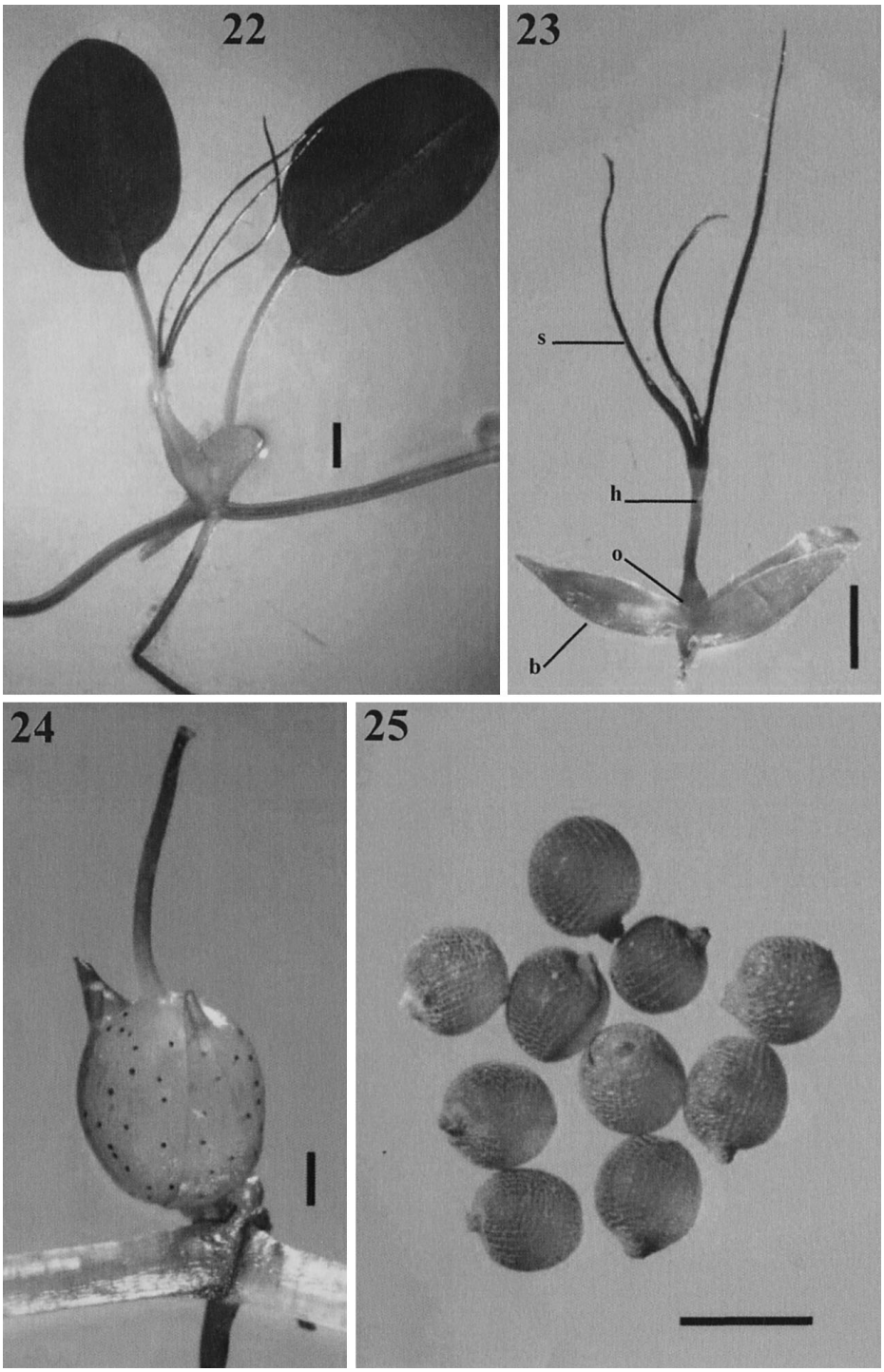
Figs. 15–16. Leaf structures of *Haophila ovalis*. (15) A young leaf pair with some branched cross veins (arrowheads) (Scale bar=2 mm). (16) Close-up view of a mature leaf (Scale bar=3 mm).

752692); 2) Ooura Bay, Okinawa Prefecture, (18.vii.2004, *leg.* Masayuki Uchimura, sterile, TNS 753978); 3) Yagachi Island, Okinawa Prefecture (19.vii.2004, *leg.* Masayuki Uchimura, pistillate, sterile and staminate, TNS 753979); 4) Sokaru, Amami-Ooshima, Kagoshima Prefecture (3.x.2004, *leg.* Kenji Sudou, sterile, TNS 753980); 5) Urasoko, Miyako Island, Okinawa Prefecture (2.xi.2004, *leg.* Shogo Arai, sterile, TNS 753981); 6) Hisamatsu, Miyako Island, Okinawa Prefecture (3.xi.2004, *leg.* Shogo Arai, sterile, TNS 753982); 7) Haemida, Iriomote Island, Okinawa Prefecture (24.xi.2004, *leg.* Masayuki Uchimura, pistillate and sterile, TNS 753983); 8) Shirahama, Iriomote Island, Okinawa Prefecture (24.xi.2004, *leg.* Masayuki Uchimura, sterile, TNS 753984); 9) Kabira Bay, Ishigaki Island, Okinawa Prefecture (25.xi.2004, *leg.* Masayuki Uchimura, sterile, TNS 753985); 10) Itona, Ishigaki Island, Okinawa Prefecture (26.xi.2004, *leg.* Tsutomu Miyazaki, sterile, TNS 753986, TNS 753987, TNS 753988); 11) Shiraho, Ishigaki Island, Okinawa Prefecture (26.xi.2004, *leg.* Shogo Arai, pistillate and sterile, TNS 753989, TNS 753990); 12) Sakieda, Ishigaki Island, Okinawa Prefecture (28.xi.2004, *leg.* Masayuki Uchimura, sterile, TNS 753991); 13) Kabila Bay, Ishigaki Island, Okinawa Prefecture (28.xi.2004; 24°26′58.6″N/124°08′53.1″E, *leg.* Masayuki Uchimura,sterile, TNS 752693, TNS 753992, TNS 753993); 14) Agonoura, Zamami Island, Okinawa Prefecture (17.xii.2004, *leg.* Masayuki Uchimura, sterile, TNS 753994, TNS 753995); 15) Aka Port, Akajima Island, Okinawa Prefecture (17.xii.2004, *leg.* Masayuki Uchimura, sterile, TNS 753996); 16) Maenohama, Akajima Island, Okinawa Prefecture (17.xii.2004, *leg.* Masayuki Uchimura, sterile, TNS 753997); 17) Eef Beach, Kumejima Island, Okinawa Prefecture (20.i.2005, *leg.* Masayuki Uchimura, sterile, TNS 753998); 18) Aara Beach, Kumejima Island, Okinawa Prefecture (20.i.2005, *leg.* Masayuki Uchimura, TNS 753999); 19) Hatenohama, Kumejima Island, Okinawa Prefecture (21.i.2005, *leg.* Masayuki Uchimura, sterile, TNS 754000); 20) Kayou, Okinawa Prefecture (19.ii.2005; 26°32′48.3″/128°06′19.3″E, *leg.* Masayuki Uchimura, staminate, TNS 752694); 21) Urasoko, Ishigaki Island, Okinawa Prefecture (7.iii.2005, *leg.* Masayuki



Figs. 17–21. Male reproductive structures of *Halophila ovalis*. (17) Young male flower with arrow indicating enveloping spathal bracts (Scale bar=2 mm). (18) and (19) Developing male flowers with arrows indicating the pedicels (Scale bars=2 mm). (20) Release of pollen (arrows) (Scale bar=2 mm). (21) Male flower after release of pollen with arrows indicating tepals (Scale bar=2 mm).

Uchimura, sterile, TNS 754001); 22) Taketomi Island, Okinawa Prefecture (12.v.2005; 24°19′21.6″N/124°04′1.02″E, *leg.* Motoya Tamaki, Takeshi Hayashibara & Masaya Katoh, staminate, TNS 752695); 23) Nakagusuku Bay, Okinawa Prefecture (31.v.2005; 26°17′14.3″N/127°52′14.5″E, *leg.* Masayuki Uchimura, pistillate, TNS 752696); 24) Nakagusuku Bay, Okinawa Prefecture (21.viii.2005, 26°17′16.5″N/127°49′12.2″E, *leg.* Masayuki Uchimura, pistillate, TNS 754002); 25) Nakagusuku Bay, Okinawa Prefecture (13.xi.2005, 26°17′21.3″N/127°50′16.5″E, *leg.* Masayuki Uchimura, pistillate, TNS 754003, TNS 754004, TNS 754005);



Figs. 22–25. Female reproductive structures of *Halophila ovalis*. (22) Female flower developing at the base of a leaf pair (Scale bar=2 mm). (23) Detail of a female flower showing enveloping bracts (b), ovary (o), hypanthium (h) and styles (s) (Scale bar=2 mm). (24) Mature fruit with several dots scattered all over the surface (Scale bar=1 mm). (25) Short-beaked seeds with enveloping coat (Scale bar=1 mm).

26) Nakagusuku Bay, Okinawa Prefecture (13.xi.2005, 26°17′26.6″N/127°50′17.0″E, *leg.* Masayuki Uchimura, pistillate and staminate, TNS 754006, TNS 754007, TNS 754008); 27) Nakagusuku Bay, Okinawa Prefecture (13.xi.2005, 26°17′31.0″N/127°50′17.0″E, *leg.* Masayuki Uchimura, sterile, TNS 754009, TNS 754010); 28) Nakagusuku Bay, Okinawa Prefecture (14.xi.2005, 26°17′32.3″N/127°50′06.2″E, *leg.* Masayuki Uchimura, pistillate, TNS 754011, TNS 754012).

Habitat and seasonality. Plants have been collected only in calm and protected areas. In the areas surveyed, they usually occurred on sandy or muddy bottoms from the intertidal zone down to about 8 m. Collection records suggest that the species is present throughout the year, although only populations from Nakagusuku Bay (Okinawa Prefecture) were regularly monitored. Occurrence of flowers and fruits was seasonal and two peaks of abundance were observed respectively in August 2005, and between November and December 2005.

Vegetative morphology. Plants were generally small with a thin, fleshy, prostrate, irregularly branched rhizome, 0.4–1 mm thick. Internodes were up to 4.5 cm long. One root and a pair of petiolate leaves were produced at each rhizome node (Figs. 13, 14). Petioles were 8–15 (–20) mm long, covered at the base by one scale. Another scale embraces the rhizome. Scales were in pair, transparent, keeled, 3–4 mm long. Leaf blades were glabrous, entire along margin, bright-green to dark-green in color, usually oval or orbicular in shape with rounded apex and base (Figs. 15, 16), (4) 6–11 (–14) mm long, (3) 4–7 (–8) mm wide, with 7–16 (–17) pairs of cross veins. Cross veins were alternate to sub-opposite or opposite along a central vein or midrib, closely spaced one another, often branched and connected to the intramarginal vein (Figs. 15, 16). The space between the intramarginal vein and blade margin was relatively wide, ranging between 0.2 and 0.4 mm (Figs. 16, 17).

Reproductive morphology. Plants were dioecious. Male or female flowers were borne at rhizome nodes enveloped by a pair of transparent spathal bracts (Figs. 13, 14). Male flowers consisted of two, 3–5 mm long spathal bracts enveloping three, 2–3 mm long tepals which in turn enclose three anther lobes containing pollen grains. They were sessile to sub-sessile at first (Fig. 17), becoming pedicellate later (Figs. 18, 19), with the pedicel extending above the spathal bracts to reach up to 20 mm in length at anthesis (Fig. 20). Pollen grains are in chains and released after opening of the tepals (Figs. 20, 21).

Female flowers were sessile or shortly stipitate and had 3 filiform styles, 15–30 mm long, prolonging a 4–6 mm long hypanthium which joins the ovary (Figs. 22, 23). The latter was oval in shape, 1.5–2.5 mm long, 0.5–2 mm in diameter. Mature fruits were light-green to yellow-white in color, ovoid to spherical, 3–4 mm long, 2.5–4 mm in diameter. In fresh state, many, small, dark-red to black dots may be visible over the pericarp of the mature fruit (Fig. 24). Seeds were globose, 0.4–0.9 mm in diameter, 4–20 in number, whitish to yellow-brown in color and shortly beaked (Fig. 25).

Molecular phylogenetic analysis

Phylogenetic analyses were based on the nuclear-encoded internal transcribed spacer (ITS1-5.8S-ITS2) of the ribosomal RNA gene and performed using MP and ML analyses. The MP analysis resulted in 10 most-parsimonious trees (500 steps, CI=0.860, RI=0.924, RC=0.795) and their strict consensus tree is presented in Fig. 27. For the ML analysis, the JC model was selected and a heuristic search was performed with the TBR branch swapping option. The single tree ($-\ln L=2969.74740$) obtained after 7975 rearrangements is presented in Fig. 28. Phylogenetic trees resulting from both analyses presented very similar topologies. The main differences between them lie in bootstrap supports for some clades (Figs. 27, 28). The samples of *H. japonica* analyzed in this study formed a strongly supported monophyletic clade (100% in MP, 99% in ML). Two sequences generated from Mugi-Ooshima (HJ3) and Mutsu Bay (HJ17) samples,

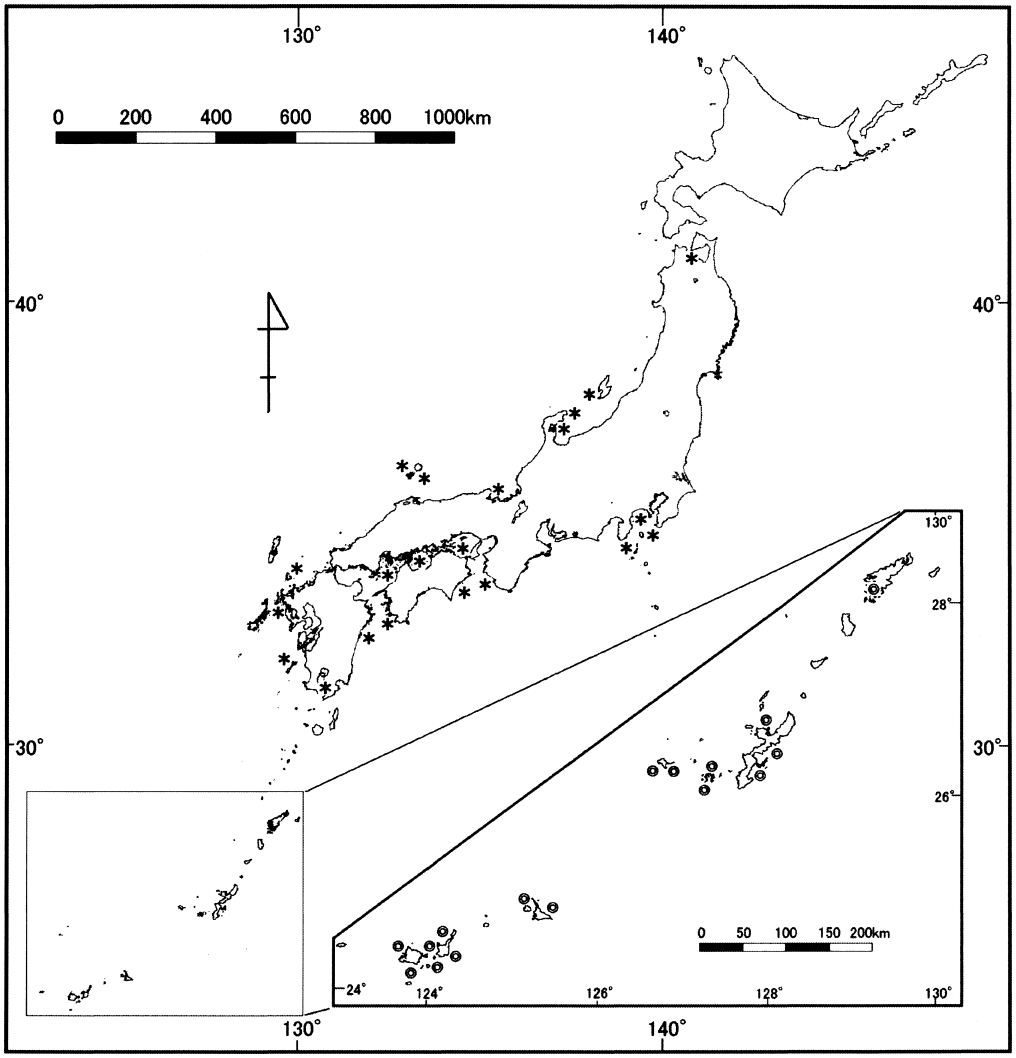


Fig. 26. Map of Japan showing distribution of *Halophila japonica* (∗) and *H. ovalis* (◎) based on recent field-collections and herbarium specimens. Inset shows details of *H. ovalis* sites in the Ryukyu Islands.

however, diverged from all remaining twelve *H. japonica* sequences by 1 bp and 2 bp respectively. *H. japonica* was resolved as sister taxon of two samples identified as *H. minor* from Guam (AF366405) and the Philippines (AF333406) respectively, with strong bootstrap support (100% in both MP and ML trees). The pairwise distances between these entities and *H. japonica* range from 9 to 15 bp (1.46–2.42% divergence) and suggest close relationships. The reference of these samples from Guam and the Philippines to *H. minor*, however, cannot be confirmed with certainty because further studies including materials from the type locality of *H. minor* are necessary. There were 24–28 bp differences (3.8–4.5% divergence) between *H. japonica* and 3 samples from Japan identified by us as *H. ovalis*, and these values suggest that they should be considered as distinct taxonomic species. In all phylogenetic trees (Figs. 27, 28) *H. japonica* and *H. ovalis* occupied distinct topological positions.

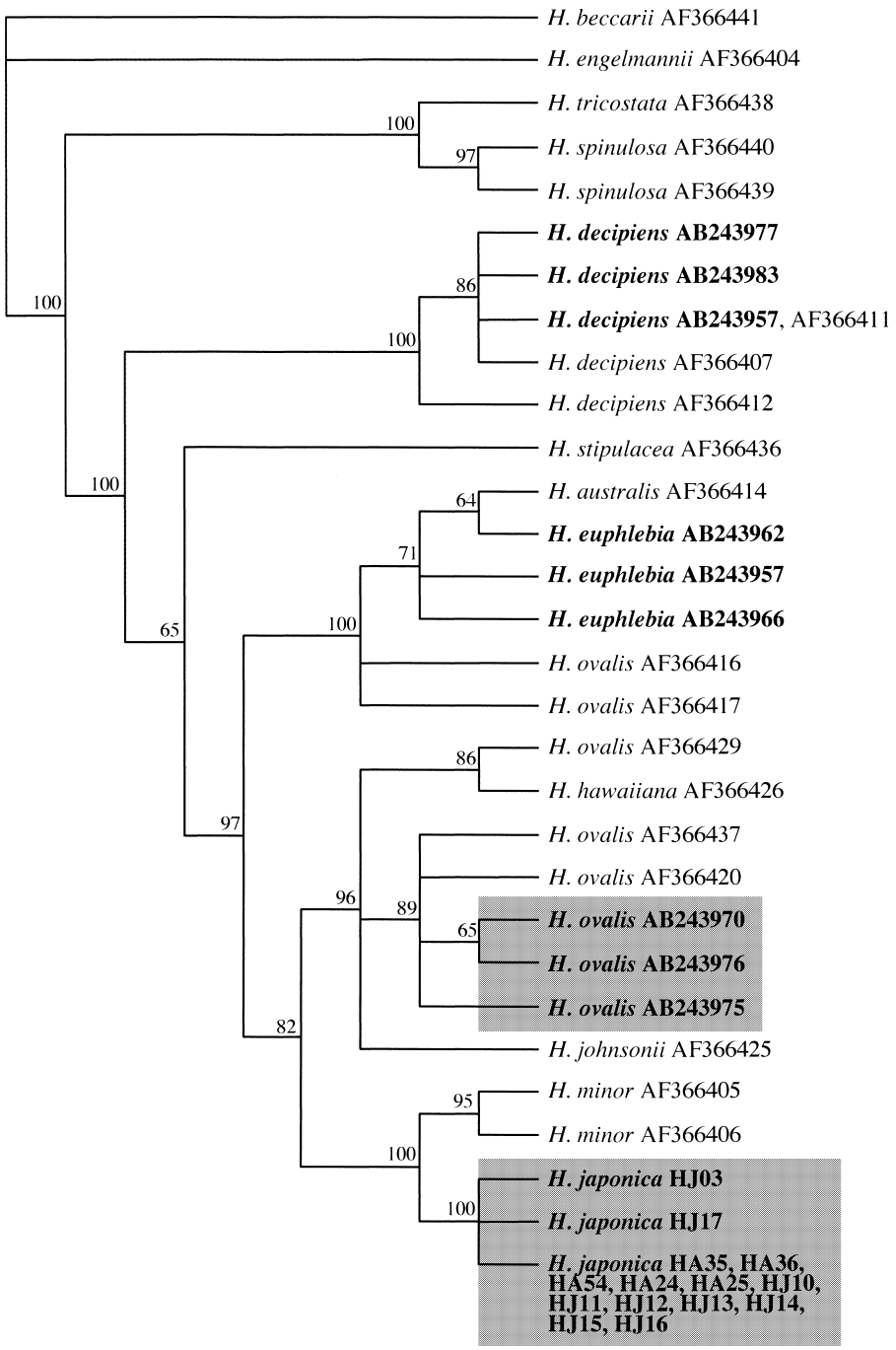


Fig. 27. *Halophila japonica*: Strict consensus tree of 10 equally parsimonious trees (500 steps, CI=0.860, RI=0.924, RC=0.795) of *Halophila* species inferred from nuclear-encoded ITS sequences. *Halophila beccarii* and *H. engelmanni* were used as outgroups. All sites were treated as unordered and equally weighted and only values above 50% bootstrap support (2000 replicates, full heuristic search with TBR method) are shown. Specimens from Japan are indicated in bold.

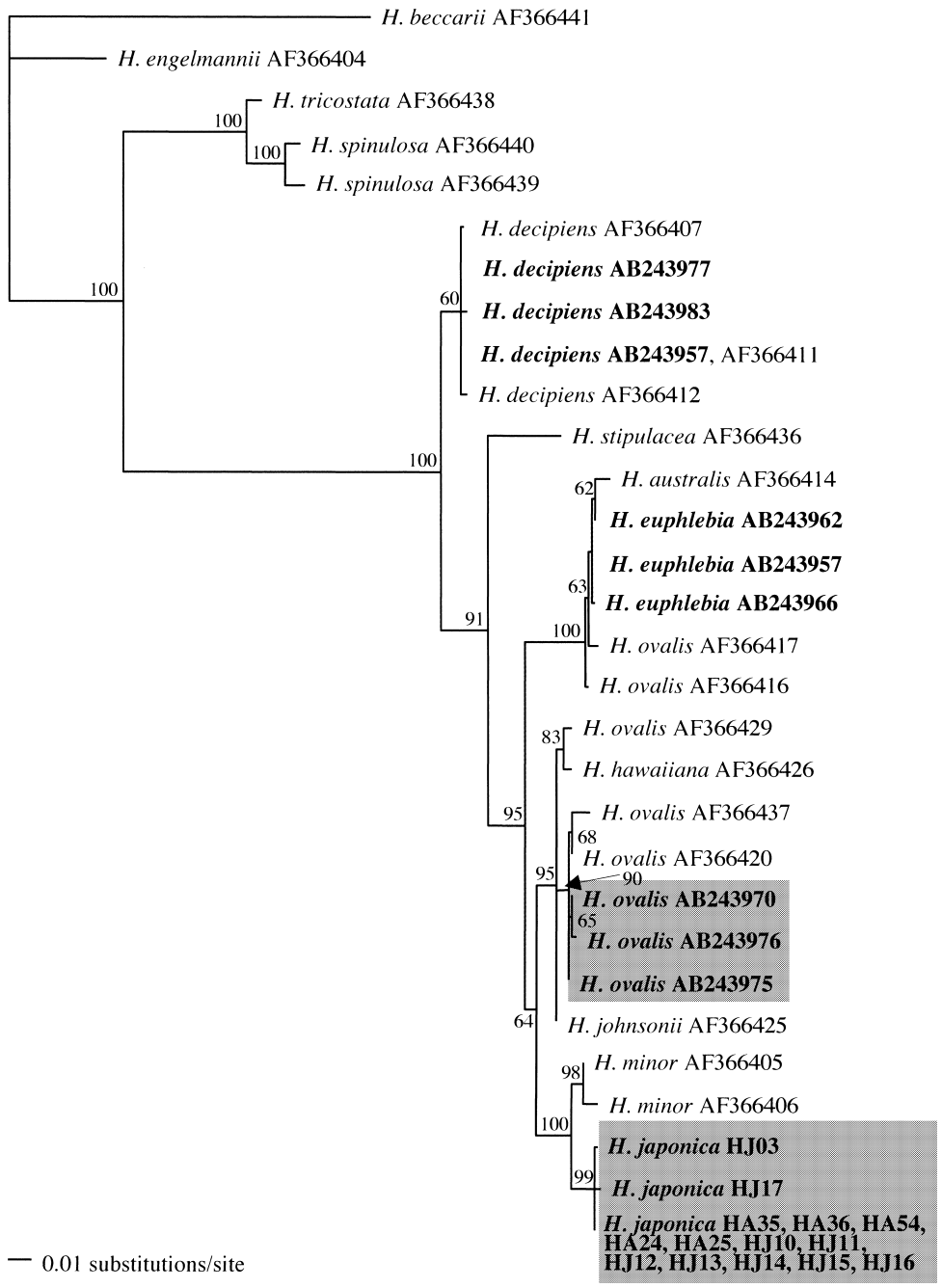


Fig. 28. *Halophila japonica*: Phylogenetic tree of *Halophila* species inferred from ML analysis (Jukes-Cantor model) of nuclear-encoded ITS sequences. *Halophila beccarii* and *H. engelmanni* were used as outgroups. Only values above 50% bootstrap support (100 replicates, full heuristic search with TBR method) are shown. Specimens from Japan are indicated in bold.

Discussion

In its vegetative organization and morphology of flowers, *Halophila japonica* fits well within the circumscription of *Halophila* sect. *Halophila* provided in den Hartog (1970), and also in Kuo and den Hartog (2001). Therefore, our morphological comparisons will be restricted to those species belonging to this grouping. At present, ten species other than *H. japonica* and the recently reinstated *H. euphlebia* Makino are assigned to section *Halophila*. These are: *H. australis* Doty et Stone, *H. capricorni* Larkum, *H. decipiens* Ostenfeld, *H. ovalis* (R. Brown) J. D. Hooker, *H. hawaiiana* Doty et Stone, *H. johnsonii* Eiseman, *H. madagascariensis* Doty et Sone, *H. minor* (Zollinger) den Hartog, *H. ovata* Gaudichaud and *H. stipulacea* (Forsskal) Ascherson (den Hartog and Kuo, 2006). The taxonomic identities of *H. hawaiiana* and *H. johnsonii*, however, require further confirmation because recent molecular studies by Waycott *et al.* (2002) could not clearly separate them from *H. ovalis*.

Moreover, although *H. ovata* materials were not available for inclusion in our molecular analyses, it seems important to emphasize that despite the fact that the name *H. ovata* was long ago shown by Sachet and Fosberg (1973) to be a superfluous name for *H. ovalis* and hence illegitimate, it is still mentioned in the literature (Kuo, 2000; Kuo and den Hartog, 2001; den Hartog and Kuo, 2006). Furthermore, Sachet and Fosberg (1973) proposed treating *H. ovata* as a synonym of *H. minor*. Therefore, in an effort to avoid further nomenclatural confusion, we think that the name *H. ovata* should not to be used again in this genus.

The reportedly pantropic species *H. decipiens* (den Hartog, 1970), and *H. capricorni* from Australia, New Caledonia and perhaps the Philippines (Larkum, 1995), are the only members of sect. *Halophila* described as being monoecious and having hairy leaves with serrulate margins. Therefore, they clearly differ from *H. japonica* which is reported here as being dioecious and having glabrous leaves with smooth margins. Another species, *H. stipulacea* is also clearly distinguished from the new taxon in that it is described as having papillose or slightly hairy, occasionally bullate leaves with serrulate margins (den Hartog, 1970; Phillips and Meñez, 1988).

In a recent taxonomic re-evaluation of the identities of two *Halophila* species (*H. minor* and *H. ovata*) that have long been confused with one another, Kuo (2000) concluded that both taxa should be regarded as distinct species, and further proposed to consider the following three morphological characters to be of diagnostic value: 1) the number of cross veins; 2) the space between two consecutive cross veins and 3) the distance between the intramarginal vein and blade margin (Kuo, 2000). The specific diagnostic characters used by Kuo (2000), together with additional ones relating to leaf and seed characteristics (leaf shape and sizes, frequency of branched cross veins, seeds diameter and number), were useful in separating *H. japonica* from all currently recognized members of the genus. A comparison of various morphological features of *H. japonica* and some related species in the section *Halophila* is given in Table 3.

Despite the fact that *H. madagascariensis* was not included in Table 3, we believe that it is clearly different from *H. japonica* in having fewer lateral veins and the presence of a small distance between the intramarginal vein and blade margin (Doty & Stone, 1967, Fig. 1B). The latter character was considered by Makino (1912) as important for discriminating *H. euphlebia* from *H. ovalis*.

In the field, particularly at Shishikui (Shikoku Island), Mugi-Ooshima (Shikoku region) and Koshiki Island (Kyushu region), the geographical distributions of *H. japonica* and *H. euphlebia* overlap as they were found growing sympatrically. Two of the voucher herbarium specimens examined in this study [(1) Tanabe, Kii Peninsula [September 1924, *leg.* Nakajima, TI (without number) and (2) Tanabe, Kii Peninsula [(month unknown) 1924, MAK 226960] also contained a mixture of both species. However, the results obtained from our detailed morphological observa-

Table 3. Comparative features of *H. japonica* and some related species in the section *Halophila* with glabrous leaves and smooth margins.

	H. australis	*H. euphlebia*	*H. hawaiiana*	*H. japonica*	*H. johnsonii*	*H. minor*	*H. ovalis*	*H. 'ovata'*
Leaf shape	Linear-lanceolate to narrow elliptical	Oval	Narrowly obovate or spatulate	Oblong to obovate	Linear	Oblong-elliptic to ovate	Oblong to ovate ovate or obovate	Oblong-elliptic,
Leaf length	5–6 cm	Up to 3 cm	2–5.3 cm	0.8–3 cm*	0.5–2.5 cm	0.6–1.2 cm	0.6–1.1 cm*	0.8–1.3 cm
Leaf width	6–15 mm	5–15 mm	1.2–6.8 mm	3–12 mm*	1–4 mm	3.5–6 mm	4–7 mm*	4–8 mm
Cross veins	14–16 pairs	12–19	10–16	6–12 pairs*	5–10 pairs	7–12 pairs	7–16 pairs*	4–8 pairs
Branched cross veins	—	Often Present	—	Rarely present	—	—	Often present	—
Distance leaf edge/ intramarginal vein	Unknown	0.1–0.2 mm	Unknown	0.2–1 mm*	0.1–0.2 mm	0.15–0.19 mm	0.2–0.4 mm*	0.4–0.6 mm
Monoecy vs. dioecy	Dioecy	Dioecy	Dioecy	Dioecy*	Probably dioecy	Dioecy	Dioecy	Dioecy
Style number	6	3	3	3 *	3	3	3	3
Style length	6–15 mm	20–40 mm	12–15 mm	6–20 mm*	Unknown	8–20 mm	10–30 mm*	6–12 mm
Seeds/fruit	50–60	13–20	12–15	5–13*	Unknown	15–30	4–20*	6–30
Seeds diameter	Unknown	0.7–1.2 mm	0.6 mm	0.4–1 mm*	Unknown	0.5 mm	0.4–0.9 mm*	Unknown
Geographical distribution	Southern Australia Tasmania New South Wales	Japan, Taiwan, Philippines	Hawai Islands	Japan	East Florida	Kenya, India, Malaysia Northern Australia	Indo-Pacific Ocean, Australia	Saipan, Guam, Micronesia Philippines
Information source(s)	Womersley 1984	Uchimura *et al.* 2006	Doty and Stone 1966, Kuo and den Hartog 2001, McDermid *et al.* 2003	This study	Eiseman 1980	den Hartog 1957 Kuo 2000, Kuo and den Hartog 2001	Womersley 1984 Phillips and Menez 1988 Kuo and Kirkman 1992 This study	Kuo 2000 Kuo and den Hartog 2001

* Data obtained from our recently collected materials.
— Not observed in this study.

tions (some of which are summarized in Table 3) show that *H. japonica* and *H. euphlebia* are clearly distinguishable on the basis of a few vegetative and reproductive characteristics including the consistency (texture) and overall shape of the leaves, the number of cross veins, the space between the intramarginal vein and blade margin, the distance between successive cross veins, and the number and size of seeds.

In Japan, *H. japonica* has long gone unrecognized and referred to as *H. ovalis* (Makino, 1912; Miki, 1934a, 1934b; Mukai *et al.*, 1980; Tanada *et al.*, 2005; Kirihara *et al.*, 2005). A close examinations of both freshly-collected materials and voucher herbarium specimens, however, revealed that it can be easily discriminated from typical *H. ovalis* species by: 1) its longer leaves, 2) its much wider space between the intramarginal vein and blade margins, as well as between two consecutive cross veins, and 3) the presence of a smaller number of cross veins which are rarely branched as opposed to those of *H. ovalis* which are more abundant and often branched (Table 3). Furthermore, although this study is not exhaustive due to limited collections, several field samplings across Japan, combined with data obtained from old collections indicate that these two species likely occupy non-overlapping geographical areas (Fig. 26), suggesting potential correlation between distributional patterns and taxonomic identity in some *Halophila* species from Japan. *H. ovalis* has been collected only from the Japanese subtropical waters (defined by Michanek (1979) as being tropical), occurring throughout the Ryukyu Islands, and extending as far north as Amami-Ooshima Island (voucher specimen TNS 753980); whereas *H. japonica* was largely found in warm temperate waters ranging between Ibusuki in the south (voucher specimen MAK 89739) and Mutsu Bay, Aomori Prefecture (voucher specimen TNS 753676) in the north (Fig. 26). These distributional separations, together with the aforementioned morphological differences were thus taken as strong supports for recognition of the entity under study as separate from *H. ovalis* with which it has been confused so far.

Sequence analyses of the internal transcribed spacer (ITS) region of the nuclear ribosomal DNA also strongly support the independent status of *H. japonica* and its establishment as a new species. In both MP and ML analyses, three representative species from Japan, namely *H. ovalis* (AB243970, AB243975, AB243976), *H. euphlebia* (AB243957, AB243962, AB243966) and *H. decipiens* (AB243977, AB243983, AB243957), did not group with *H. japonica* and were placed in separate clades (Figs. 27, 28). *H. japonica* and two samples identified as *H. minor* from Guam (AF366405) and the Philippines (AF333406) formed a strongly supported clade (100% bootstrap support in both MP and ML trees), which allied with weak to moderate support (64% in ML, 82% in MP) to another clade comprising *H. hawaiiana* (AF366426), *H. johnsonii* (AF366425) and *H. ovalis* from Australia (AF366429), Malaysia (AF366420), Japan (AB243970, AB243975, AB243976) and Vietnam (AF366437). Except for the inclusion of new ITS sequences of *Halophila* materials from Japan, our results are very reminiscent of those of Waycott *et al.* (2002) which, on the basis of ITS sequence analyses demonstrated that *H. minor* constitutes a distinct species in this genus. However, although the independent status of the samples referred to as *H. minor* by Waycott *et al.* (2002) is strongly supported in this study (95% bootstrap support in MP, 98% in ML), their taxonomic placement still remains uncertain because although Sachet and Fosberg (1973) earlier treated *H. ovata* sensu Gaudichaud from Guam and the Philippines as conspecific with *H. minor*, recent studies by Kuo (2000) provided evidence that *H. minor* is a different species. Ostenfeld (1909) had recognized *H. ovata* sensu Gaudichaud as a species distinct from *H. ovalis* but applied to it the illegitimate name *H. ovata*. This name is still being used for plants reported from Saipan, Guam, Yap and Manila Bay in the Philippines (Kuo and den Hartog, 2001; den Hartog and Kuo, 2006). Consequently, further comparative morphological and molecular studies includ-

ing material from the type locality (Lesser Sunda Islands, Flores, Indonesia) (Kuo, 2000) of *H. minor* are clearly needed to confirm the true identity of the plants from Guam and the Philippines currently going under the name of *H. minor*.

Nevertheless, our results reported in Table 3 clearly suggest that both *H. minor* and *H. japonica* can be distinguished on the basis of a few morphological characteristics of which, the distance between the intramarginal vein and the blade margin [0.2–1 mm in *H. japonica* (This study) vs. 0.15–0.19 mm in *H. minor* (Kuo, 2000)] is, in our opinion, reliable enough to serve for their discrimination. Based on such morphological feature, the two species become easily separable from one another as, for example, *H. ovalis* is from *H. euphlebia* (Makino, 1912; Uchimura *et al.*, 2006), or *H. 'ovata'* (though this name is illegitimate according to Sachet and Fosberg, 1973) is from *H. minor* (Kuo, 2000; Waycott *et al.*, 2004). Additionally, the two species seem to be geographically isolated because *H. minor* is so far reported to occur in tropical regions only (Sachet and Fosberg, 1973; Kuo, 2000; den Hartog and Kuo, 2006), whereas *H. japonica* is presently known in the Japanese warm temperate region only; its highest latitude being Mutsu Bay in the northern part of Honshu. Taking all these molecular, morphological and geographical distribution differences into consideration, we therefore conclude that *H. japonica* should be considered as independent and thus strongly merits a specific status in this genus. In addition, since the materials from Guam and the Philippines which are currently very likely to be mistakenly treated as *H. minor* (Waycott *et al.*, 2002) appeared to be closely related to *H. japonica* on the basis of ITS sequence data (Figs. 27, 28), we predict that they may represent southern variants of our newly described species from Japan. However, more comparative morphological studies are required to clarify this hypothesis.

It should be further pointed out that *Halophila* populations from Mutsu Bay (Aomori Prefecture, Japan) currently represent the northernmost limit of distribution for this seagrass genus, and this was recently reported by Kirihara *et al.* (2005). On the basis of recent collections from the same locality (Table 1), however, we propose that the newly established name *H. japonica* be applied to Kirihara *et al.* (2005) material, which was recorded under *H. ovalis*.

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