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Re-evaluation of the distribution of *Monactinus biwaensis* based on quantitative morphological analysis of extant and fossil specimens

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Abstract: *Monactinus biwaensis* (Negoro) Matsuoka et Kayama was described as a new species in 1954 as *Pediastrum biwae* Negoro from Lake Biwa in Central Japan. This species is characterized by projections from adjacent marginal cells that either face each other or diverge. However, there was also the view that *P. biwae* was not an independent species but rather a variety of *P. simplex* as *P. simplex* var. *biwaense*. Subsequently, no other water systems yielded *Pediastrum* species with this morphology, and *P. biwae* was long considered endemic to the Lake Biwa–Yodo River system. Measurement of the side ratio of marginal cells in *Monactinus simplex* collected from Lake Biwa and molecular phylogenetic analysis revealed that *M. biwaensis* and *M. simplex* are clearly distinguishable. This result strongly suggests that two morphologically similar species, *M. simplex* and *M. biwaensis* occur in Lake Biwa at present. Fossils of *M. biwaensis* have been found in the Ayama Formation of the Late Pliocene Kobiwako Group and in the Ma0 Member of the Early Pleistocene Osaka Group, leading to the assumption that this species was endemic to Lake Biwa–Yodo River System. However, *M. biwaensis* was also found in the Middle Pleistocene Karato Formation on Himeshima Island of Oita Prefecture and in the Sahama Mud Member along the coast of Lake Hamana, which are distant from the Lake Biwa–Yodo River System. In addition to this, *M. biwaensis* has also been observed in surface sediments from Harima Nada and Isa Bay, both located outside the Lake Biwa–Yodo River System. Furthermore, *M. biwaensis* occurs in other countries outside Japan, primarily in the Southeast Asian region. Taken together, these findings clearly indicate that *M. biwaensis* is not endemic to the Lake Biwa–Yodo River System.

Key words: Karato Formation, Lake Biwa–Yodo River System, *Monactinus simplex*, non-endemic species, *Pediastrum biwae*, Sahama Mud Member

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Introduction

Various palynomorphs, which are composed of organic-walled microfossils, are preserved in lake and marine sediments. The *Pediastrum* s.l. group is one of these palynomorphs and is used to reconstruct paleo sedimentary environments. This group, previously collectively termed *Pediastrum*, has been subdivided into at least six genera (*Pediastrum* Meyen, *Stauridinium* Gorca, *Monactinus* Gorca, *Parapediastrum* E.Hegerald, *Pseudopediastrum* E.Hegerald, *Lacnastrum* H.A.McMnus) based on recent molecular phylogenetic and morphological analyses (McManus et al. 2011, McManus & Lewis 2005). The genus *Monactinus*, characterized by a single projection on its marginal cells of coenobia, is also a member of *Pe-*

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diastrum s.l., and consists of *Monactinus simplex* (Meyen) Corda, *M. biwaensis* (Negoro) Matsuoka & Kayama, and *M. asymmetricus* (Yamagishi & E.Hegewaid) E.Hegewald (Guiry & Guiry 2016). *M. biwaensis* was first described as a new species in 1954 by Negoro as *Pediastrum biwae* originating from Lake Biwa. This species is characterized by projections from adjacent marginal cells that either face each other or diverge. However, several authors considered *P. biwae* not an independent species but rather a variety of *Pediastrum simplex* Meyen, as *P. simplex* var. *biwaense* (sic) (Fukushima 1956, Naselli-Flores & Barone 2011). Subsequently, no other drainage systems yielded *Pediastrum* s.l. with this morphology, and so *Pediastrum biwae* Negoro was long regarded as endemic to the Lake Biwa–Yodo River system, until recently. Re-examining reports identifying specimens from Korea (Kim & Kim 2012), the Philippines (Martinez-Goss et al. 2016), Thailand (Prasertsin et al. 2018), Malaysia (Khaw et al. 2020), India (Naidu et al. 2018), and Brazil (Loaiza-Restano 2013) as *Pediastrum simplex* based on the characteristic "opposing or diverging projections from adjacent marginal cells", it became clear that all these specimens could be considered *P. biwae* without contradiction (Kayama et al. 2025). Furthermore, when a phylogenetic tree was constructed including the Malaysian specimen for which molecular phylogenetic data had been published, it became clear that this specimen belongs to the clade of *M. biwaensis* (Kayama et al. 2025). However, these were only occurrence reports and did not provide any biogeographical background.

In the paleontological sense, *Pediastrum* s.l. are one conspicuous member of the aquatic palynomorphs and have been recovered as fossils from various strata of different ages (e.g., Wilson & Hoffmeister 1953, Nasu 1971, Singh & Khanna 1976, Matsuoka & Hase 1977, Harada 1984, Zamaloa & Tell 2005, El Noamani & Saleh 2018). *Pediastrum biwae* has also been recorded as fossils from the Pliocene Kobiwako (Paleo-Biwa) Group in Japan (Nasu 1971). However, it is necessary to confirm the morphological characteristics of the fossil *M. biwaensis* in order to correctly identify the species using the morphometric characteristics mainly of its marginal cells. For this purpose, we collected surface sediments from Lake Biwa, Osaka Bay, Harima-Nada of the Seto Inland Sea, and Ise Bay and examined morphological changes after palynological processing, because these *Pediastrum* s.l. fossils can survive after palynological treatment using HCl and HF. Also, *M. biwaensis* has been thought to be found only in Lake Yogo (located north of Lake Biwa), Lake Biwa, in ponds and marshes near Nara, and in ponds and marshes in Senriyama of Osaka, and these localities were considered to belong to the Lake Biwa–Yodogawa River System of the central part of Japan from a viewpoint of paleodrainage connectivity (Negoro 1959, Nasu 1971).

In this paper, we will also consider whether *M. biwaensis* is endemic to the Lake Biwa–Yodo River System or not based on morphometric data of marginal cells of fossils collected from surface and consolidated sediments and molecular phylogenetic analysis.

Materials and Methods

Sampling location

Plankton samples yielding *Monactinus biwae* and *M. simplex* were collected at several different sites in Lake Biwa using a plankton net with 20 μm or 41 μm mesh size.

The surface sediment samples were collected with a gravity TFO corer type B (Rigosha, Tokyo, Japan) and an Ekman-Birge bottom sampler (Rigosha, Tokyo, Japan) from sites in Lake Biwa, Osaka Bay, Harima-Nada of the Seto Inland Sea and Ise Bay (Table 1). The consolidated sediments, referred to the Karato Formation in Himeshima Island, Oita Prefecture were also provided for extracting palynomorphs including fossil *Pediastrum* s.l.

The Karato Formation is composed with the marine and freshwater sediment layers accompanied with several tephra beds (Naya et al. 2024), and is contained within the Early to Middle Pleistocene in terms of geologic age (Mizuno 2018). This formation is divided into lower, middle and upper parts (Naya et al. 2024) and several mud samples were collected from the marine and freshwater mud layers located in the middle parts.

Living specimens of *Monactinus simplex* were collected from Nishinoko of Lake Biwa and *M. simplex* var. *echinulatum* (Wittrock) Pérez, Maidana & Comas from the ecological observation pond at the Lake Biwa Museum. The strain of *M. simplex* was established using this collected specimen assigned the identifier SeedBank 25000-1.

These sampling locations are shown in Fig. 1 and Table 1.

Palynological analysis

For understanding how *Pediastrum* s.l. algae remain preserved in sediments as fossils, the top 2 cm of the sediment cores collected off western Chikubu-shima Island in Lake Biwa, southwest of the mouth of Yodo River of Osaka Bay and south of the mouth of Ichikawa River of Harima Nada, and the center of Ise Bay were used for extracting palynomorphs, including *Pediastrum* s.l. remains using HCl (Hydrogen Chloride, ca. 10% in concentration) and HF (Hydrofluoric acid, ca. 36% in concentration) at room temperature for ca. 24 hours. The procedure for extracting palynomorphs from the consolidated sediments of the Karato Formation followed the steps below. The samples were pulverized and dispersed using KOH (potassium hydroxide) treatment, followed by removal of silicate minerals via HF treatment. Subsequently, to concentrate plant matter, density separation was performed using a $ZnCl_2$ (Zinc chloride) solution with a specific gravity of approximately 1.9. After CH_3COOH (Acetic acid) treatment, cellulose was decomposed and removed via acetolysis treatment (adding a mixture of concentrated H_2SO_4 (sulfuric acid) and $(CH_3CO)_2O$ (Anhydrous acetic acid) in a 1 : 9 ratio,

Table 1. Sampling location and sample type.

Sampling site		Alphabet in Figure 1	Sample type	Sampling device	Sampling date	Reference
Lake Biwa	Ecological observation pond in the Lake Biwa Museum	A-1	Plankton	Plankton net (41 μm mesh size)	October 16, 2025	This study
	Orura Fishing Port	A-2	Plankton	Plankton net (20 μm mesh size)	June 10, 2022	Kayama et al. 2025
	Nishinoko	A-3	Plankton	Plankton net (41 μm mesh size)	November 7, 2022	This study
	Off western Chikubu-shima	A-2	Surface sediment	TFO type B corer	October 21, 2021	Kayama et al. 2025 & This study
		A	Surface sediment			Nasu 1971
Southwest of the mouth of Yodo River	Osaka Bay	A-3	Surface sediment	TFO type B corer		This study
South of the mouth of Ichikawa River	Harima Nada	A-4	Surface sediment	Ekman-Birge sampler		This study
Center of the bay	Ise Bay	A-5	Surface sediment	Ekman-Birge sampler		This study
Toyonaka, Osaka Prefecture	Senriyama	C	Ma 0, Osaka Group	outcrop		Nasu 1971
Off Senshu, Osaka Prefecture	Kansai Air port	C	Ma 8?, Osaka Group	core sediments		Harada 1983
Northern part of Nara Prefecture	Saho Kyuryo	C	Ma 1, Osaka Group	outcrop		Matsuoka 1972
Off Kunisaki peninsula, Oita Prefecture	Himeshima	D-6	Karato Formation	outcrop		This study
Western part of Shizuoka Prefecture	Lake Hamana	D-7	Sahama Mud Member	outcrop		Matsuoka & Hase 1977
South part of Shiga Prefecture	Yubune Tuff	B	Lower member, Kobiwako Group	outcrop		Nasu 1971

followed by heating in a water bath for 1 minute). After neutralizing with pure water, refined materials for light microscopic (LM) study were prepared by sieving with a screen mesh with 10 μm opening size. When broken coenobia were encountered, these specimens were recorded if these were identified as each species using the criteria in this article.

Observation under light microscope

From plankton and sediment samples, living and fossil coenobia of *Monactinus biwaensis* and *M. simplex* were observed with an upright NIKON ECLIPSE E600 light microscope (Nikon, Japan) and photographs were taken using a NIKON Digital Camera DS-5Mc (Nikon, Japan).

Morphological analysis of fossil materials

Since the morphology of the marginal cell is one of the important characteristics for differentiating *M. biwaensis* and *M. simplex* (many of these algae were originally named *Pediastrum simplex*), we measured the length of sides of marginal cells to determine the morphometric characteristics of these species, as shown in Figs. 2 and 3. Since *M. simplex* and *M. biwaensis* have a single projection, the outer edges of the projection and the marginal cell were combined to form two side lines. The lengths of these two lines were measured to obtain the ratio of the two sides (hereafter called "side ratio"). However, because the sides facing the projection of *M. biwaensis* and *M. simplex* var. *echinulatum* were bent in the middle, they were divided into two parts as shown in Fig. 3 (-4, -6). To determine the ratio per coenobium, at least three peripheral cells were measured and the average value was calculated.

Fossil coenobia did not usually show a complete morphology like planktonic living forms. When coenobia were broken, deformed and bent, marginal cells properly preserved were selected for morphometric measurement. In such cases, measurement of the side ratio was less than three cases as shown in Fig. 4 (-1, -3, -4, -5, -6). For clarifying the morphological differences between *M. simplex* and *M. biwaensis*, the ratio of sides of marginal cells of these fossil species were measured and analyzed. The side ratio of marginal cells of *M. simplex* coenobia of plankton in Lake Biwa (Fig. 2 [-1, -2, -3]) and fossil coenobia from the surface sediments of Osaka Bay (Fig. 4-1) were measured. For *M. biwaensis*, the length of sides of marginal cells of the specimens preserved in surface sediments of Lake Biwa, Osaka Bay, Harima-Nada and Ise Bay were measured (Fig. 4 [-3, -4, -5, -6]). Fossil *M. biwaensis*

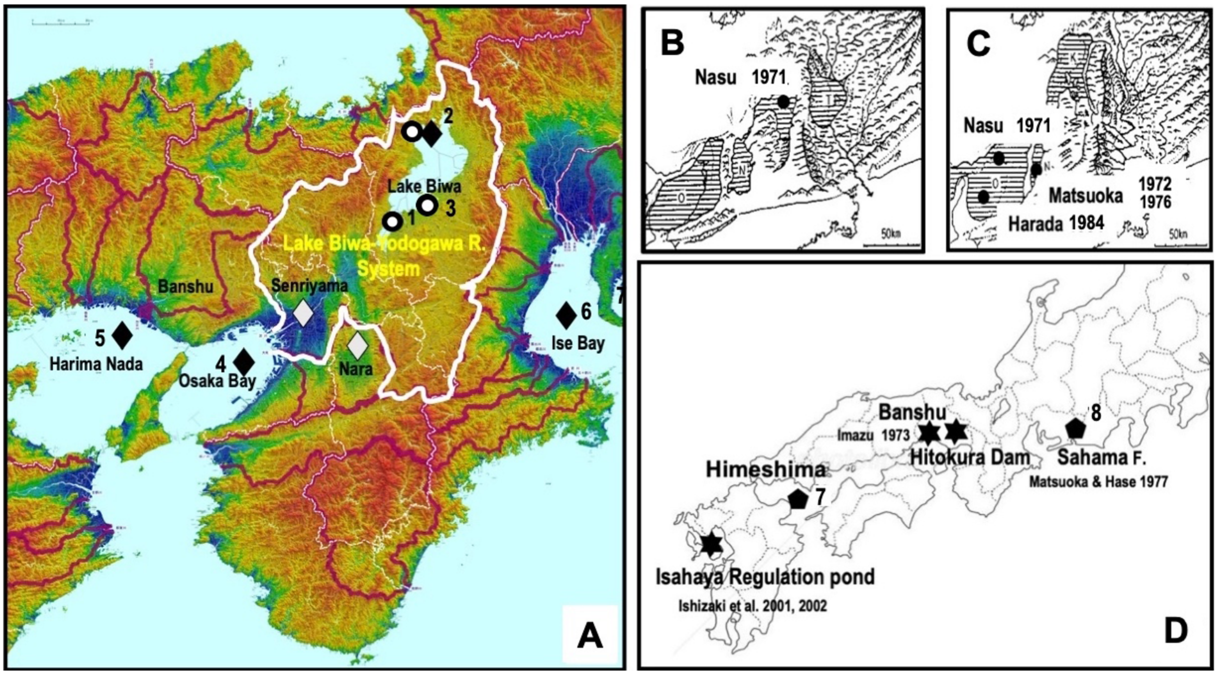

Fig. 1. Sampling locations and occurrence reports of *Monactinus biwaensis* and *M. simplex* in the present study. A, white line showing the area of the drainage of the Lake Biwa–Yodo River System, Locations of the ecological observation pond in the Lake Biwa Museum (1), Oura Fishing Port (2), and Nishinoko (3) ◉; Locations of surface sediments (4–6) ◆; Locations of reported occurrence of fossil *Monactinus* ◇. B, Occurrence site of fossil *M. biwaensis* in the Kobiwako Group (Nasu 1971) on the paleo-geographic maps of Sugiyama (1991). C, Occurrence sites of fossil *M. biwaensis* and *M. simplex* in the Osaka Group (Nasu 1971, Matsuoka 1972; 1976, Harada 1984) on the paleo-geographic maps of Sugiyama (1991). D, Occurrence sites of *Monactinus biwaensis* outside of the Lake Biwa–Yodo River system ✶, 7 Occurrence sites of fossil *M. biwaensis* from the Karato Formation in Himeshima Island, Oita Prefecture ⬟. 8 Occurrence sites of fossil *M. biwaensis* of Sahama Mud Member from Lake Hamana, Shizuoka Prefecture ⬟.

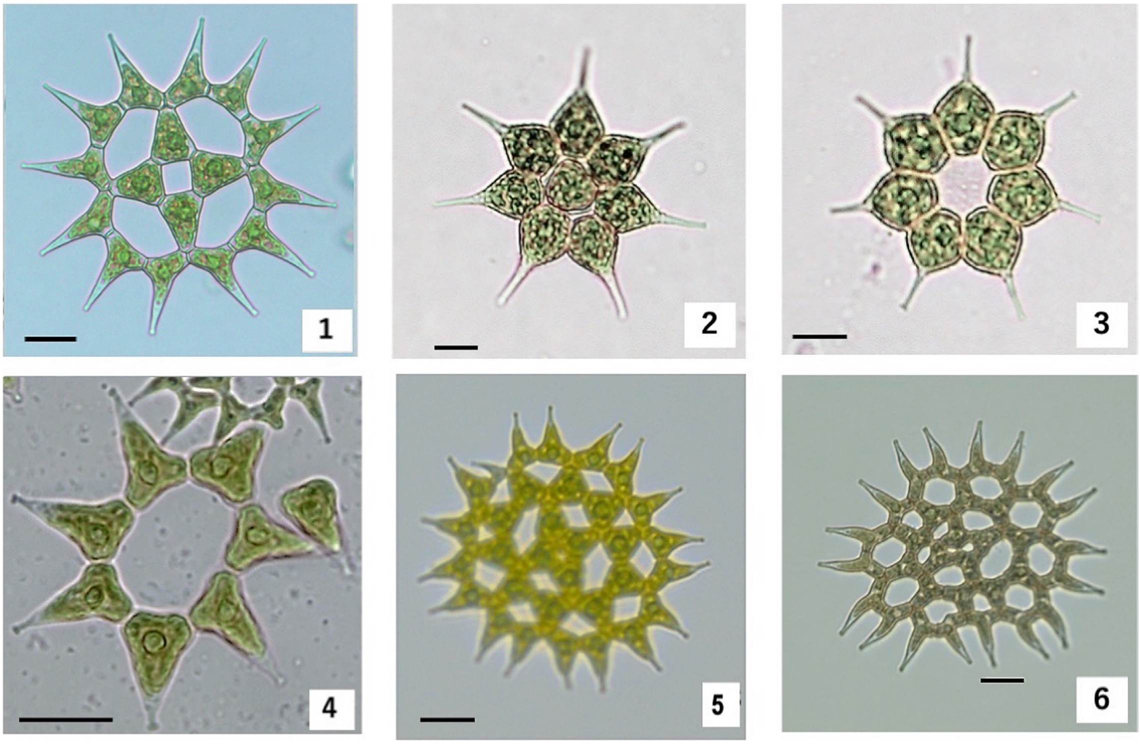

Fig. 2. 1, Living plankton identified as *Monactinus simplex* collected from Nishinoko, Lake Biwa, 2–3; *Monactinus simplex* var. *echinulatum* collected from the ecological observation pond in the Lake Biwa Museum, 4–6, Living plankton of *Monactinus biwaensis* collected at Oura Fishing Port, northern part of Lake Biwa. Scale bar 20 μm.

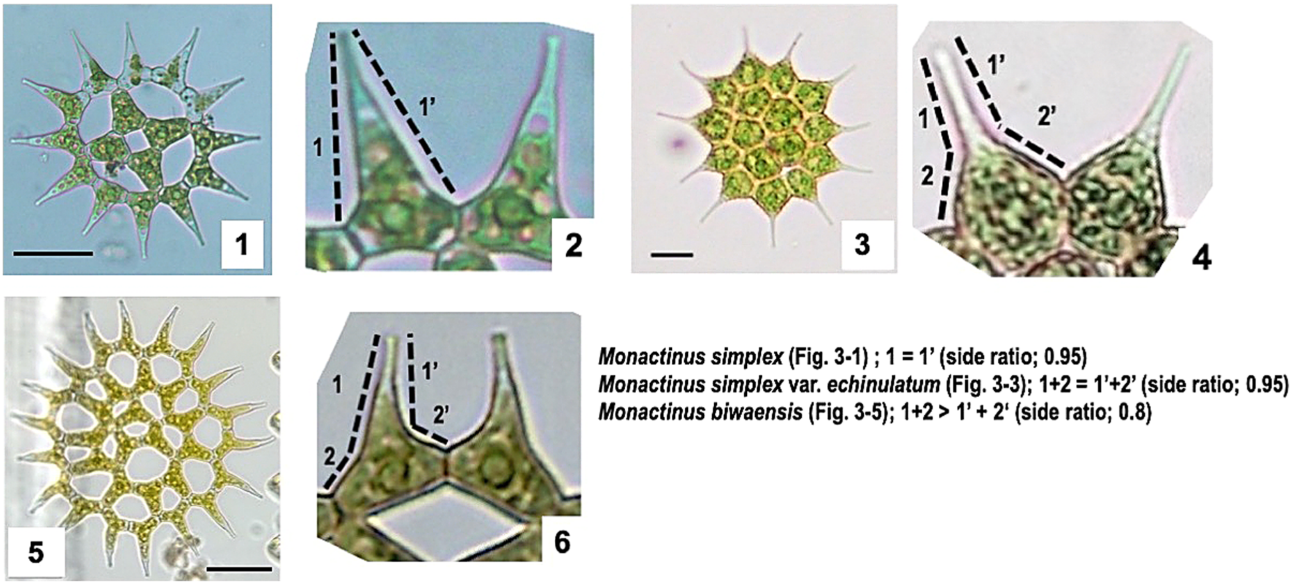

Fig. 3. Specimens from which the side ratios were measured. 1; *Monactinus simplex* (Plankton) collected from Nishinoko, Lake Biwa, 2; Lines measured on marginal cells of *Monactinus simplex*, 3; *Monactinus simplex* var. *echinulatum* (Plankton) collected from the ecological observation pond in Lake Biwa, 4; Lines measured on marginal cells of *Monactinus simplex* var. *echinulatum*, 5; *Monactinus biwaensis* (plankton) collected from the Oura Fishing Port, northern part of Lake Biwa, 6; Lines measured on marginal cells of *Monactinus biwaensis*. Scale bar 20 μm.

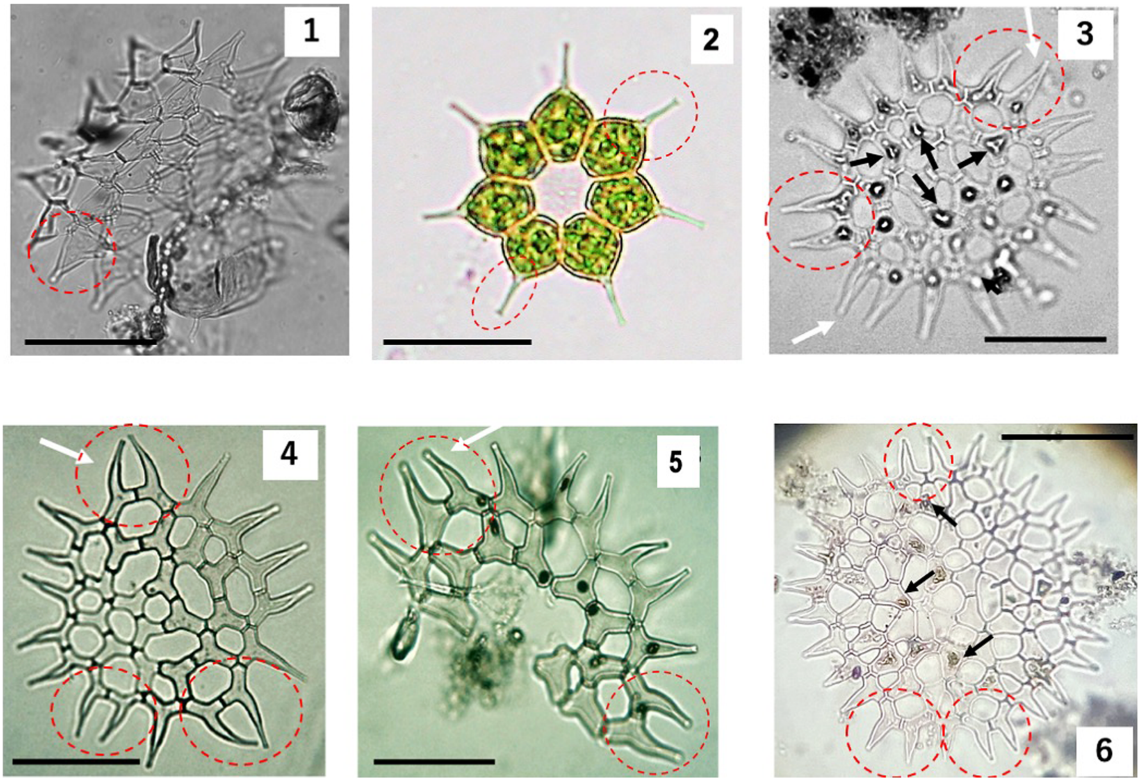

Fig. 4. Measurement of the side ratio of marginal cells indicated with dotted ellipsoidal or circular lines, 1; *Monactinus simplex* deformed (fossil, surface sediment of Osaka Bay), 2; *Monactinus simplex* var. *echinulatum* (living form, Ecological observation pond of Lake Biwa Museum), 3; *Monactinus biwaensis*, arrows indicating shrunk protoplasts (fossil, surface sediment of Harima Nada), 4; *Monactinus biwaensis* (fossil, surface sediment of Lake Biwa), 5; *Monactinus biwaensis* fragmented (fossil, surface sediment from Osaka Bay), 6; *Monactinus biwaensis*, arrows indicating shrunk protoplasts (fossil, surface sediment from Ise Bay). Scale bar 20 μm.

Fig. 5. Fossil specimens of *Monactinus biwaensis* collected from the Early to Middle Pleistocene Karato Formation on Himeshima Island, Oita Prefecture. Dotted ellipsoidal lines showing characteristics of marginal cells of *Monactinus biwae*. 1; 220111-1 D11 U34/2, 2; 220111-1 D11 P32/4, 3; 220111-1 D11 J39/2, 4; 220111-1 D11 E37/3, positions on the mount slide (220111-1 D11) were indicated with England Finder. Scale bar 20 μm.

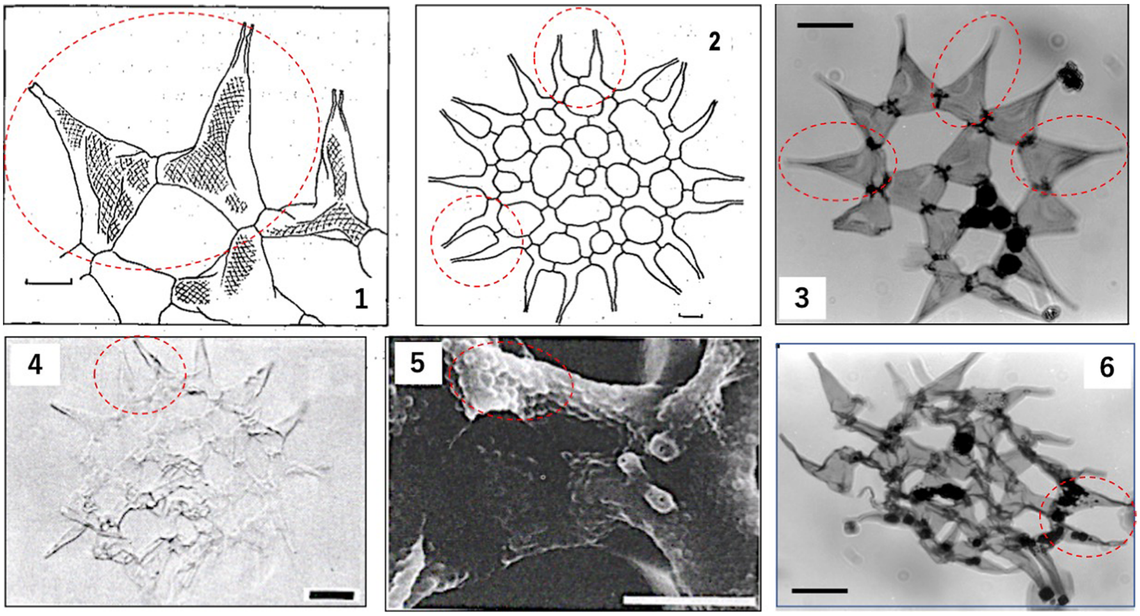

Fig. 6. Fossil specimens of *Monactinus biwaensis* reported from various locations. 1; Illustration of *Monactinus biwaensis* (as *Pediastrum biwae*) from Ma0 Member of the Osaka Group (Fig. 2-1, 2 of Nasu 1971). 2; Illustration of *Monactinus biwaensis* (as *Pediastrum biwae* var. *triangulatum*) from the surface sediments of Lake Biwa (Fig. 2-2, 3 of Nasu 1971). 3; *Monactinus simplex* from the Ma1 Member of the Osaka Group, north of Nara City (Fig. 12 of Matsuoka 1972), 4; *Monactinus biwaensis* (as *Pediastrum biwae*) from the Ma8? Member of the Osaka Group (Pl. 3, fig. 1 of Harada 1984), 5; *Monactinus biwaensis* (as *Pediastrum biwae*) from the Ma8? Member of the Osaka Group (Pl. 3, fig. 2 of Harada 1984), SEM photograph showing granular ornaments (dotted ellipsoidal line), 6; *Monactinus biwaensis* from the Ma1 Member of the Osaka Group, north of Nara City (Fig. 3 of Matsuoka 1972). Dotted circular or ellipsoid lines showing characteristics of marginal cells of *M. biwaensis* and *M. simplex*. Scale bar 10 μm except for Fig. 5-5. Scale bar 5 μm for Fig. 5-5.

observed in the Karato Formation were also provided for morphometric analysis (Fig. 5). Several studies that recorded *M. biwaensis* (as *P. biwae*) have been published accompanied with some microphotographs and illustrations [Nasu 1971 (Fig. 6 [-1, -2]), Matsuoka 1972 (Fig. 6 (3, -6)), Harada 1984 (Fig. 6-4), Matsuoka & Hase 1977 (Fig. 7)]. The side ratio of marginal cells of these fossil coenobia were also measured, when properly shown in the photographs as suggested above.

DNA extraction and molecular phylogenetic analysis

Some specimens of *Monactinus simplex* uniquely identified as SeedBank 25000-1 (Fig. 2-1) that were collected from Nishinoko in Lake Biwa were used for molecular phylogenetic analysis. Total DNA of the strain was extracted using a Wizard® Genomic DNA Purification Kit (Promega Co. Ltd., USA) following the manufacturer's instructions. PCR amplifications were performed using KOD-plus-Ver2 (TOYOBO Co. Ltd., Osaka, Japan) and two primer sets: LSU (26S) rDNA gene (5′-GCATATCAA

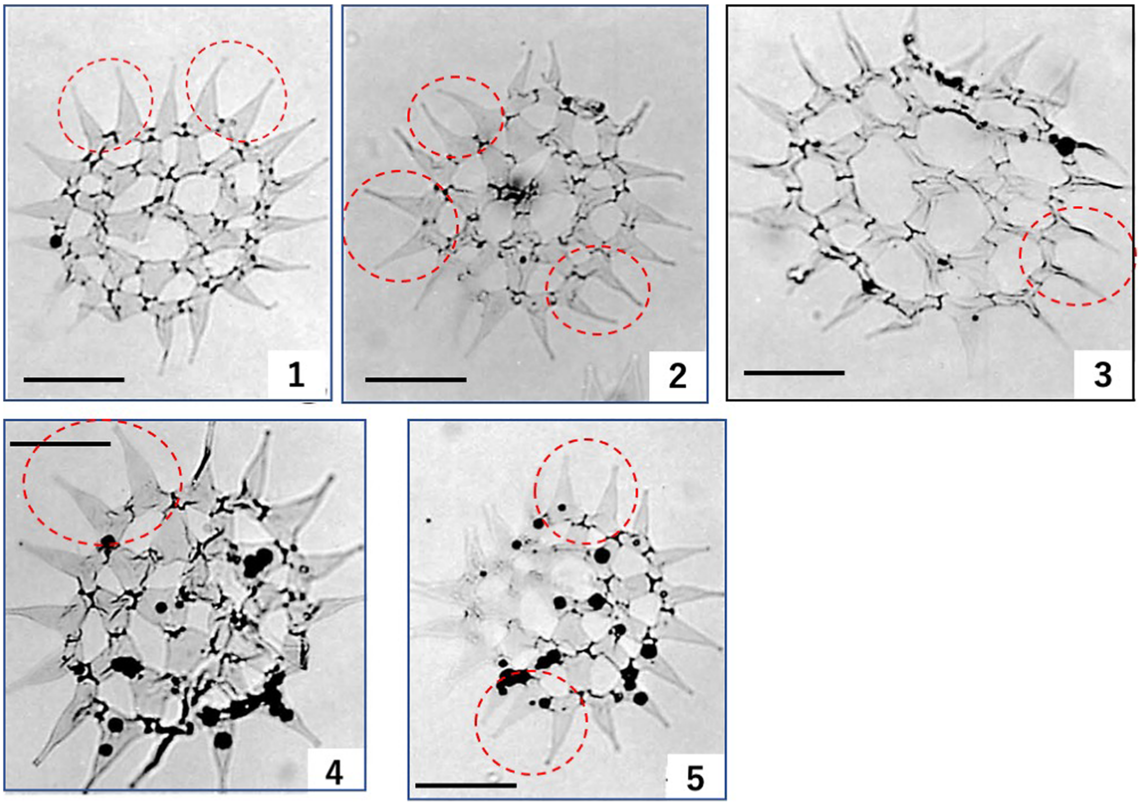

Fig. 7. Fossil specimens re-observed from the Sahama Mud Member of the Hamamatsu Formation. 1; *Monactinus biwaensis* (as *Pediastrum simplex* var. *duodenarium* (Reproduction from Pl. 46, Fig. 6 in Matsuoka & Hase 1977), 2; *Monactinus biwaensis* (=*Pediastrum simplex* var. *duodenarium* (Reproduction from Pl. 46, Fig. 8 in Matsuoka & Hase 1977), 3; *Monactinus biwaensis* (as *Pediastrum simplex* var. *duodenarium* (Reproduction from Pl. 46, Fig. 11 in Matsuoka & Hase 1977), 4; *Monactinus simplex* (=*Pediastrum simplex* var. *duodenarium* (Reproduction from Pl. 46, Fig. 7 in Matsuoka & Hase 1977), 5; *Monactinus simplex* (=*Pediastrum simplex* var. *duodenarium* (Reproduction from Pl. 46, Fig. 12 in Matsuoka & Hase 1977). Dotted ellipsoidal or circular lines showing characteristic marginal cells, the side ratios of which were measured in this study. Scale bar 20 *µ*m. Reproduction from Pl. 46, Figs. 6, 7, 8, 11, 12 in Matsuoka & Hase 1977, with permission from The Palaeontological Society of Japan. (© The Palaeontological Society of Japan.)

TAAGCGGAGGA-3′, 5′-TCCCCTTGTCCGTACCAGT-3′; Buchheim et al. 2005), and internal transcribed spacer 2 (ITS2) region gene (5′-CGATACGTAGTGTGAATTGC-3′, 5′-GAGGGTACTTCTATAGACTACAATTCTCC-3′; Kayama et al. 2025). PCR amplification was conducted for 30 cycles with a denaturation step at 96°C for 30 s, an annealing step at 46°C for 20 s, and an elongation step at 68°C for 5 min.

For the strain of *Monactinus simplex*, the LSU rDNA and ITS2 region were examined. These gene sequences were aligned with available sequence data for the Hydrodictyaceae extracted from the GenBank nucleotide database using MAFFT (Katoh & Standley 2013). Ambiguously aligned sites were removed manually using BioEdit (Hall 1999). The LSU rDNA and ITS2 region dataset comprising 17 taxa and 2161 sites were used for constructing phylogenetic trees. Three datasets were subjected to phylogenetic analysis using IQ-TREE 3.0.1 (Nguyen et al. 2015), under the TN+F+I+G4 model with 100 bootstrap replicates.

Results

The relationship between *Monactinus biwaensis* and *Monactinus simplex* based on the molecular phylogenetic trees

Monactinus simplex collected from Nishinoko in Lake Biwa for determination of the LSU rDNA and ITS2 regions were provided to make phylogenetic trees together with three strains of *M. biwaensis* (GenBank Accession No. LC740460, LC773701, LC740459, LC773703, LC740458, and LC773703). As a result, *M. simplex* was included in the cluster of *Monactinus*, as shown in Fig. 8. The *Monactinus simplex* clade was clearly separated from *M. biwaensis*. In the *M. simplex* clade, two subclades were formed the *M. simplex* SeedBank 25000-1Subclade and the *M. simplex* UTEX Subclade.

In the molecular phylogenetic tree, the *Monactinus* clade was differentiated from the other clades of *Pediastrum* s.l. Within the *Monactinus* clade, *M. biwaensis* and *M. simplex* (SeedBank 25000-1, UTEX LB1601 and UTEX 1601) were clearly separated (bootstrap value=100%; Fig. 8).

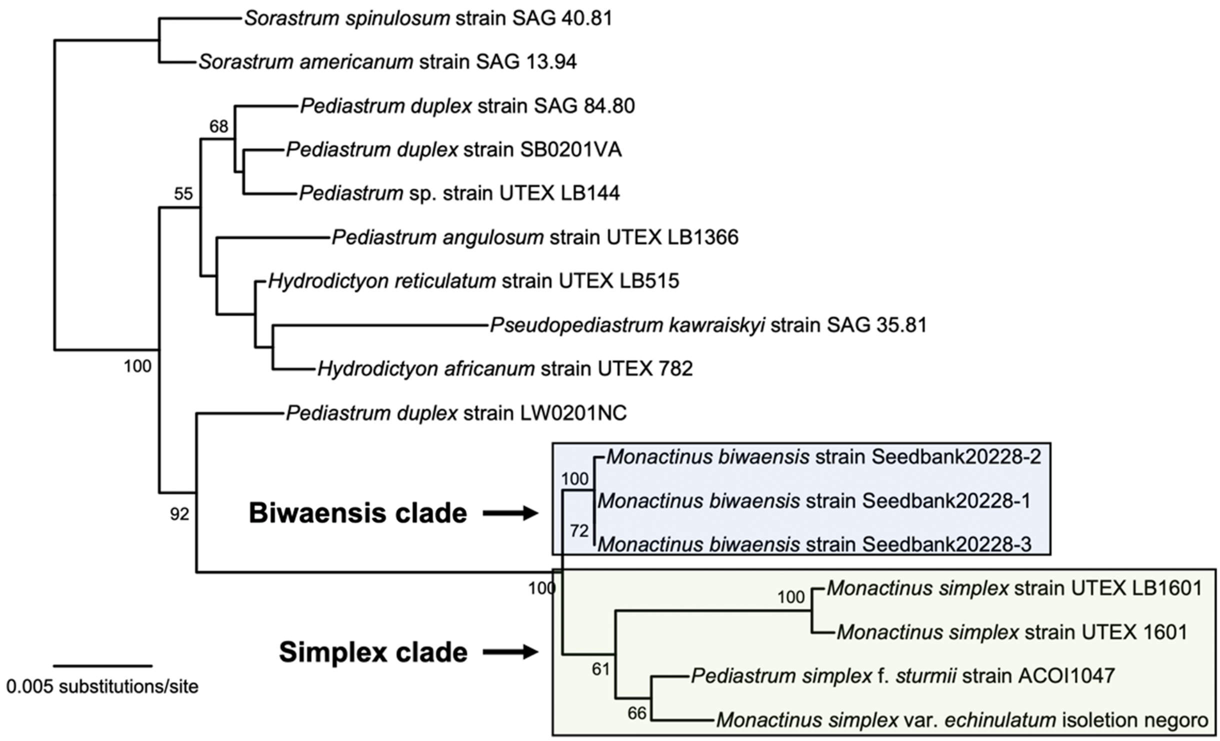

Fig. 8. Maximum likelihood tree of LSU rDNA and ITS rDNA gene sequences of *Monactinus* species. The tree was inferred with IQ-TREE 1.6.12 under the TN+F+I+G4 model. Bootstrap values ≥50 are shown on branches. Light yellow color indicates the Biwaensis clade and Light green color indicates the Simplex clade.

Consequently, *M. biwaensis* and *M. simplex* are also considered independent species from each other based on the molecular phylogenetic analysis. The *M. simplex* strain (SeedBank 25000-1) is also different from the *M. simplex* strains (UTEX LB1601 and UTEX 1601).

Remains of *Pediastrum* s.l. preserved in sediments after palynological analyses

After removing calcareous and silicate sediment particles palynologically, various palynomorphs, including coenobia of *Monactinus biwaensis*, were observed in the surface sediments listed in Table 1. Fossil *Pediastrum* s.l. were also observed without any size differences between two the different methods used for extracting from the unconsolidated surface sediments and consolidated sediments from the Karato Formation.

Off the western coast of Chikubu Island, several chlorophyceaen fossils like *M. biwaensis*, *M. simplex*, *Xanthidium hastiferum* var. *javanicum* (Nordst) Turner, *Tetraedron gracile* (Reinsch) Hansgirg, and *Staurastrum dorsidentiferum* var. *ornatum* Grönblad were preserved in the surface sediments. The samples from Osaka Bay contained various chlorophycaean fossils, *M. biwaensis*, *M. simplex*, *Pediastrum duplex* Meyen, *Staurastrum doesidentiferum* var. *ornatum*, *Desmodesmus* sp., *Senedesmus* sp., and *Botryococcus braunii* Kützing accompanied with various marine and freshwater palynomorphs. In the samples from Harima Nada, a few chlorophycean fossils, *M. biwaensis*, *M. simplex* and *Staurastrum* sp. were observed. In the surface sediments from Ise Bay, several chlorophycean fossils including *M. biwaensis, M. simplex, Pseudopediastrum boryanum* (Tyrpin) E.Hegewald, *Desmodesmus* sp., *Senedesmus* sp., and *Staurastrum* sp. were also found. However, except for the samples from Lake Biwa, the density of these chrolophycean fossils was rather low. The densities of the coenobia of *M. biwaensis* were observed as follows; Osaka Bay was 1002 coenobia/g of dry sediment, Harima Nada 47 coenobia/g of dry sediment, 8 coenobia/g of dry sediment from Ise Bay, and off western Chikubushima in Lake Biwa 5045 coenobia/g of dry sediment. Some of these coenobia were deformed and broken, but the morphological characteristic of the orientation of the adjacent projections from the marginal cells (i.e., whether they face each other or are separated) was preserved even in those specimens as shown in Fig. 4 (-3, -4, -5, -6).

Side ratio of the marginal cells of *Monactinus biwaensis* and *M. simplex*

The chemicals used to extract palynomorphs containing *Pediastrum* s.l. from modern surface sediments and consolidated sediments from the Karato Formation and the Sahama Mud Member differed, so it could not be ruled out that the extracted palynomorphs vary in size. However, the side ratio of the marginal cells used to distinguish *Monactinus biwaensis* and *M. simplex* based on morphological characteristics follows a consistent ratio, meaning it is not affected by changes in coenobia size.

The side ratios for the plankton specimens of *M. biwaensis* and *M. simplex* were 0.8 and 0.95, respectively (Fig. 3). The side ratios for coenobia of *M. biwaensis* obtained for surface sediments ranged from 0.64 to 0.85 (Average 0.74, n=6) for Ise Bay, 0.77–0.89 (Average 0.85, n=6) for Harima Nada, 0.74–0.82 (Average 0.78, n=6) for Osaka Bay, and 0.68–0.8 (Average 0.76, n=6) for Lake Biwa. The side ratio of marginal cells of the fossil coenobia of *M. biwaensis* recorded in Plio-Pleistocene sediments ranged from 0.62 to 0.97 (Average 0.82, n=19) for the Karato Formation, 0.77–0.96 (Average 0.83, n=10) for the Sahama Mud Member, 0.71–0.95 (Average 0.83, n=2) for the Ma0 Member of Osaka Group, and 0.85–0.86 (Average 0.86, n=2) of the Ma8? Member of the Osaka Group. On the other hand, the side ratios for *M. simplex* were 0.95–0.97 (Average 0.96, n=6), *M. simplex* var. *echinulatum* 0.89–1 (Average 0.95) for Lake Biwa (plankton), 1 (n=1) for a specimen preserved in the surface sediments of Osaka Bay, 1–0.87 (Average 0.96, n=5) for the Pleistocene Sahama Mud Member, and 0.94 (Average 0.94, n=3) for the Ma1 Member of the Osaka Group (Table 2).

Discussion

Legitimacy of *Monactinus biwaensis*

Monactinus biwaensis (=*Pediastrum biwae*) was once

Table 2. Ratio of the two sides of the marginal cells of living and fossil *Monactinus biwaensis* and *Monactinus simplex*. The average of the side ratio of living *M. simplex* and *M. biwaensis* collected from Lake Biwa, the type locality, is significant with a p-value<0.01.

Side ratio of marginal cells of *Monactinus biwaensis* in plankton		
Kayama et al. 2025		0.73–0.88
Side ratio of marginal cells of *Monactinus biwaensis* in surface sediments		
Isa Bay (n=6)		0.74
Harima Nada (n=6)		0.85
Osaka Bay (n=6)		0.78
Lake Biwa (n=6)		0.76
Range		0.74–0.85
Side ratio of marginal cells of fossil *Monactinus biwaensis*		
Karato Formation (n=19) (Eraly to Middle Pleistocene, This study)		0.82
Sahama Mud Bed (Middle Pleistocene, Matsuoka & Hase 1977)		
Pl. 46 Fig. 6 (n=4)		0.86
Pl. 46 Fig. 8 (n=3)		0.85
Pl. 46 Fig. 11 (n=3)		0.8
Karato Formation (n=19) (Eraly to Middle Pleistocene)		0.82
Osaka Group (n=2) (Ma0; Late Pliocene, Nasu 1972)		0.83
Osaka Group (n=2) (Ma8?; Middle Pleistocene, Harada 1984)		0.86
Range		0.8–0.86
Side ratio of marginal cells of *Monactinus simplex* including var. *echinulatum* in plankton		
Kayama et al. 2025		0.97
Lake Biwa (n=3)	*Monactinus simplex* var. *echinulatum*	0.96
Lake Biwa (n=3)	*Monactinus simplex*	0.95
Range		0.95–0.97
Side ratio of marginal cells of *Monactinus simplex* in surface sediments		
Osaka Bay (fossil) n=1		1
Range		1
Side ratio of marginal cells of fossil *Monactinus simplex*		
Sahama Mud Bed (Middle Pleistocene, Matsuoka & Hase 1977)		
Pl. 46 Fig. 7 (n=4)		0.92
Pl. 46 Fig. 12 (n=1)		1
Osaka Group (Ma1, Early Pleistocene, Matsuoka 1976)		0.94
Range		0.92–1

considered to be a variety of *P. simplex* as *Pediastrum simplex* var. *biwaense* (sic) even before its description as a new species (Mizuno 1952, Fukushima 1956). Furthermore, even after its description, it has been noted that some specimens hardly observed the characteristic morphological feature of *Monactinus biwaensis* projections of marginal cells that either face each other or are separated. Tanaka (2022, p. 588) mentioned that intermediate forms between *M. biwaensis* and *M. simplex* sometimes inhabit Japanese freshwater fields. However, the method to measure the ratios of the marginal cells can affect the quantification of this characteristic morphology. In plankton samples collected from the ecological observation pond at the Lake Biwa Museum, a different morphotype characterized by having short and fine spines on the cell surface of *M. simplex* was observed. This morphotype is identical to *Monactinus simplex* var. *echinulatum* and its side ratio values were 0.89–1. Therefore, the side ratios of *M. simplex*, including *M. simplex* var. *echinulatum* and *M. biwaensis*, were measured as 0.95 and 0.8 average, respectively. From the view point of the molecular phylogenetic analysis, *M. biwaensis* and *M. simplex* formed distinct clades in the *Monactinus* lineage as shown in Fig. 8.

In conclusion, *M. biwaensis* and *M. simplex* (SeedBank 25000-1) are independent species based on both the morphological and molecular phylogenetic analyses.

Lake Biwa–Yodo River System

Lake Biwa receives inflows from the Yogo River, which originates from Lake Yogo to the north, as well as the Ane River, the Yasu River, the Ado River, the Hino River, and the Echi River. Lake Biwa's waters flow out through the Seta River, the Uji River, and then the Yodo River into Osaka Bay. Along this route, the Uji River, the Katsura River, and the Kizu River converge in the southwestern Kyoto Basin to form the Yodo River. Therefore, the Lake Biwa–Yodo River system refers to the watershed that encompasses Lake Biwa and the Yodo River and their tributaries, including Shiga Prefecture; the Iga-Ueno Basin in Mie Prefecture; the Muro Mountains and Yamato Highlands in Nara Prefecture (for the Kizu River); the Kyoto and Kameoka basins in southern Kyoto Prefecture (for the Katsura River); northeastern Osaka Prefecture (for the Yodo River); and the eastern part of Hyogo Prefecture. However, when discussing whether *M. biwaensis* might be endemic to Lake Biwa or not, attention should be focused on the sediments preserved in the basins along the Seta River, the Uji River, and the Kizu River. This is because the drainage basin evolution of ancient Lake Biwa, which connects to the present-day lake, unfolded within these three river basins. The lower part of the Ueno Formation of the Kobiwako Group was formed in the Ueno Basin in the upper Kizu River, the middle part of the Gamo Formation of the Kobiwako Group was formed along the present-day Yasu and Echi Rivers, and the upper part of the Katata Formation was formed in an aquifer of the Ado River (Satoguchi 2017). Therefore, examining the strata preserved in the paleo-basins of the Seta, Uji, Kizu, Yazu, Ado, and Echi rivers is crucial for understanding the provenance of *M. biwaensis.*

Occurrence of *Monactinus biwaensis* in Japan

Pediastrums biwae as *M. biwaensis* has been reported from waters of the Lake Biwa–Yodo River system (Negoro 1959, Nasu 1971). According to Negoro (1959), the drainage system around Lake Biwa is as follows: Lake Yogo, the Yogo River, Lake Biwa, the Seta River, the Uji River, the Yodo River and finally into Osaka Bay as shown above. However, *M. biwaensis* has also been listed from the following locations: southwestern part (Banshu) of Hyogo Prefecture (Imazu 1973), Regulation Pond of Isahaya Reclamation in Nagasaki Prefecture (Ishizaki et al. 2001, 2002), and Hitokura Dam (Japan Water Agency Kansai Yoshinogawa Branch Yodogawa Headquarters Hitokura Dam Management Office 2020) at present. Although these occurrence reports were not accompanied by any descriptions, photographs, or drawings, but rather were only included in lists of species, these lists suggested that *M. biwaensis* is distributed outside of the Lake Biwa–Yodo River system. The Plankton Guidebook provided by the Institute of Biology of Yokkaichi University suggests that *M. biwaensis* (*P. biwae*), which was previously regarded as an endemic species to the Lake Biwa–Yodo River System, is now found in various water bodies nationwide (https://web.yokkaichi-u.ac.jp/bio/zukan/pediastrum-biwae/). However, this statement needs further explanation, because the recent expansion of *M. biwaensis* in Japan is probably not natural but rather due to anthropogenic activities. Since more than one hundred years ago, larvae of sweetfish (Ayu) inhabiting Lake Biwa have been commercially moved to other regions (Imura 2013). At that time, the larvae of sweetfish were transported together with the water of Lake Biwa, which possibly contained small phytoplankton including, *M. biwaensis*.

Occurrence of *Monactinus biwaensis* outside of Japan

There are several reports of the occurrence of *Pediastrum biwae* outside of Japan. The following specimens seemed to be identical to *M. biwaensis* (=*P. biwae*); Korea (Kim & Kim 2012), the Philippines (Martinez-Goss et al. 2016), Cambodia (Yamagishi & Hirano 1973), Thailand (Prasertsin et al. 2014), north-eastern coast of India (Jena & Adhikary 2011), the south-eastern coast of India (Naidu et al. 2018), Turkey (Baykal et al. 2004), and San Paulo district of Brazil (Loaiza-Restano 2013) (Fig. 9). Among these reports, a Malaysian strain shown in Fig. 4 F6 by Khaw et al. (2020) is noteworthy, because a morphological feature and molecular phylogenetic analysis was performed. The side ratio of marginal cells of the Malaysian strain is nearly 0.88, which suggests this strain is identical to *Monactimus simplex* or *M. biwaensis.* Actually, Khaw et al. (2020) finally identified this strain as *P. simplex*. How-

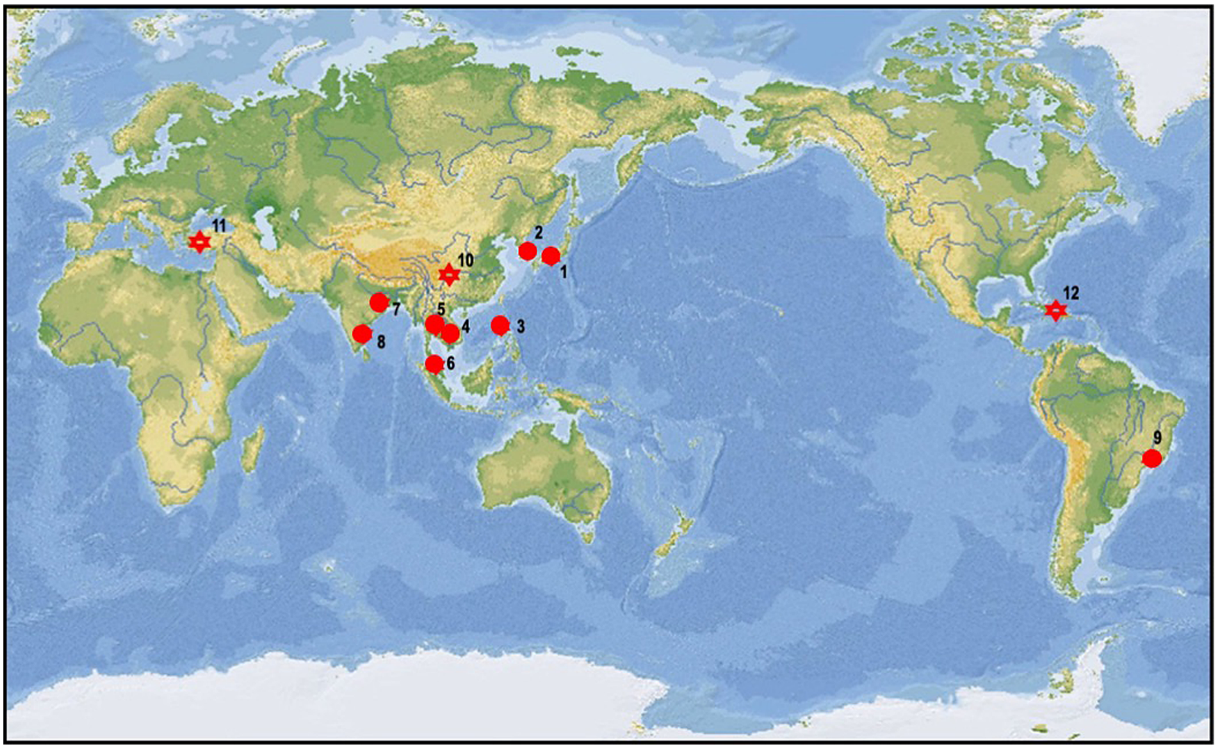

Fig. 9. Distribution of *Monactinus biwaensis* (as *Pediastrum biwae*) in the world including possible records without any illustrations. ●: Drawings or photographs, ✡: Species name only. 1 Japan; Negoro (1954), 2 Korea; Kim & Kim (2012), 3 Philippines; Martinez-Goss et al. (2016), 4 Cambodia; Yamagishi & Hirano (1973), 5 Thailand; Prasertsin et al. (2014), 6 Malaysia; Khaw et al. (2020), 7 North-eastern India; Jena & Adhikary (2011), 8 South-eastern coast of India; Naidu et al. (2018), 9 Brazil; Loaiza-Restano (2013), 10 China; Xiang et al. (2021), 11 Turcky as *M. simplex* var. *duodenarium*; Baykal et al. (2004), 12 Cuba; González (2009).

ever, the Blast analysis suggested this strain (accession no. MH166739) was identical to *Lacunastrum gracillimum* (West & G.S.West) H.A.McManus rather than *P. simplex*. However, using the data stored in GenBank (accession no. MH166739), Kayama et al. (2025) constructed a phylogenetic tree based on SSU rDNA and showed that this strain was clearly included in the *Monactinus biwaensis* Clade. Consequently, *M. biwaensis* has also been observed in several places outside of Japan.

Occurrence of *Monactinus biwaensis* preserved in surface sediments in Japan

The surface sediments off Chikubu shima Island in Lake Biwa contain numerous *M. biwaensis* cells and remains in excellent condition as mentioned previously. The side ratio of their marginal cells is 0.76 on average. On the other hand, Nasu (1971) illustrated *M. biwaensis* (as *P. biwae*) extracted from Lake Biwa surface sediments; measuring the side ratio of marginal cells in that illustration yields 0.8. Therefore, the side ratio of marginal cells in *M. biwaensis* in the sediment of Lake Biwa ranges from 0.8 to 0.76. Some coenobia of *M. biwaensis* were also recovered from surface sediments in Osaka Bay. The average side ratio of marginal cells of the specimens of Osaka Bay was 0.78, which also falls within the range for this species in Lake Biwa.

Monactinus biwaensis preserved in surface sediments from Harima Nada and Ise Bay appeared in low concentrations, and their marginal cells were small, making it somewhat difficult to confirm the characteristic facing or separating arrangement of marginal cells. This seems to be due to the small size of individual cells, as these coenobia consist of 32 to 64 cells in total. Even so, the average side ratios of the marginal cells were 0.74 and 0.85, respectively, which are nearly identical to those of *M. biwaensis* from the surface sediments of Lake Biwa. Although only one specimen of *M. simplex* was observed in the surface sediments of Osaka Bay, the side ratio of this specimen was 1 (Fig. 4-1). Therefore, *M. simplex* and *M. biwaensis* preserved in surface sediments at various locations can also be distinguished based on the side ratio of their marginal cells.

Since Osaka Bay is directly affected by the Lake Biwa–Yodo River system, the rather high abundance of *M. biwaensis* remains is not surprising. On the other hand, the very low density of *M. biwaensis* is reasonable due to Harima Nada and Ise Bay being clearly outside the Lake Biwa–Yodo River system. The presence of *M. biwaensis* in these areas indicates that the remains were most likely transported by the rivers flowing into Harima Nada and Ise Bay form ponds and/or lakes nearby. Moreover, since these specimens had contracted cytoplasm, it is highly likely that this specimen was deposited immediately on the bottom of Harima Nada, possibly via the Ichikawa River System and Ise Bay (Fig. 4-3, -6). In the case of Harima Nada this is consistent with the report by Imazu (1973) that *P. biwae* (=*M. biwaensis*) was growing in the Banshu Plain. Therefore, *M. biwaensis* is not endemic to the Lake Biwa–Yodo River system based on this evidence.

Fossil record of *Monactinus biwaensis* preserved in the Middle Pleistocene sediments in Japan

Fossil *Monactinus biwaensis* was also observed from several different sites in Japan. Nasu (1971) reported the occurrence of *M. biwaensis* cited as *P. biwae* and *P. biwae* var. *triangulatum* from several formations, including the mud layer (Ayama Formation of the Kobiwako Group) 17 m below Yubune Tuff (Earliest Pleistocene) in Shiga Prefecture, the Ma0 Member of the Osaka Group at Senriyama in Osaka Prefecture, and the Ma1 Member of the Osaka Group (Middle Pleistocene) at Takano-hara area in Nara Prefecture.

Harada (1984) reported that the granulate structure on the cell surface was also preserved on the cell surface of fossil *Pediastrum* sp. from the Ma 7 or 8 Member of the Pleistocene Osaka Group (Middle Pleistocene) suggested by Nakaseko & Nishiwaki (1984). The SEM observation of *M. biwaensis* preserved in the modern sediments of Lake Biwa showed granules on the cell surface. On the other hand, *M. simplex* has reticulated ornaments with spines on trigonal or tetragonal mesh (Fig. 11 in Kayama et al. 2025). This fine morphological feature suggests that the *Pediastrum* sp. of Harada (1984) may be identical to *M. biwaensis*. However, these fossil occurrences from the Plio-Pleistocene Kobiwako Group and Osaka Group do not directly prove that *M. biwaensis* is not an endemic species to the (paleo-) Lake Biwa–Yodo River System, because these occurrences are within the (paleo-) Lake Biwa–Yodo River System.

On the other hand, fossils of *M. biwaensis* have also been reported from several locations outside the present-day Lake Biwa–Yodo River System. These fossils were recorded from the Karato Formation on Himeshima Island in Oita Prefecture and the Sahama Mud Member along the coasts of Lake Hamana, Shizuoka Prefecture. From the Karato Formation, *M. biwaensis, M. simplex* and *Pseudopediastrum boryanum* (Raciborski) Lenarczyk have also been observed. The average side ratio of fossils of *M. biwaensis* from the Karato Formation was 0.82 on average, indicating they belong to *M. biwaensis* rather than *M. simplex.* According to Mizuno (2018) and Naya et al. (2024), the depositional age of the Karato Formation is the Early to Middle Pleistocene.

Matsuoka & Hase (1977) reported the discovery of *Pediastrum* s.l. fossils from the Sahama Mud Member of the Middle Pleistocene Hamamatsu Formation which deposited in the coastal area of paleo Lake Hamana. From the Sahama Mud member, fossil *Pediastrum* s.l. were identified as *Pediastrum araneosum* var. *rugulosum* (G.S. West) G.M. Smith, *Pediastrum duplex* var. *coharens* Bohlin, *Pediastrum boryanum* Meneghini, and *Pediastrum simplex* var. *duodenarium* (Bailey) Rabenhorst, and *Pediastrum simplex* var. *inflatum* Matsuoka (Matsuoka & Hase 1977). Noteworthy variants were *P. simplex* var. *duodenarium*, and *P. simplex* var. *inflatum*. This variety was considered a variety of *Pediastrum simplex*, distinct from *Pediastrum biwae* var. *ovatum*, because the projection marginal cells of var. *inflatum* did not face each other or diverge (Pl. 46, figs. 1–5 in Matsuoka & Hase 1977). *P. simplex* var. *duodenarium* has a more elongated marginal cell than *P. simplex* var. *inflatum*, and in some specimens, the projections of the marginal cells were observed to either face each other or diverge (Pl. 46, figs. 6, 8, 11 in Matsuoka & Hase 1977, but some specimens lacked such pronounced morphological features (Pl. 46, figs. 7, 9 in Matsuoka & Hase 1977). Therefore, Matsuoka & Hase (1977) identified these specimens as *P. simplex* var. *duodenarium*. At that time, the characteristics of the projection of the marginal cell had not been quantified and examined, so in this study, the side ratio of the marginal cell was newly measured for these specimens (Fig. 7). The results ranged from 0.86 to 0.80, which is slightly larger than the side ratio for the marginal cells of *M. biwaensis* collected from Lake Biwa and Osaka Bay, but still falls within the range of the side ratio of *M. biwaensis* reported to date. As a result, the specimens shown in Fig. 7-1, -2, -3 (=Pl. 46, figs. 6, 8, 11in Matsuoka & Hase 1977) from the Sahama Mud Member may be identified as *M. biwaensis.*

Matsuoka (1972) reported the occurrence of *M. simplex*, *Psudopediastrum boryanum*, and *Monactinus* sp. from the Ma1 Member of the Osaka Group in the Takano-hara area of Nara Prefecture. *Monactinus* sp. (Fig. 6-6) was poorly preserved, making it impossible to measure the side ratio of the marginal cell. However, given its characteristic slender and asymmetrical marginal cell, it is highly likely to be *M. biwaensis*. This suggests that *M. simplex* and *M. biwaensis* might coexist even in the Ma1 Member of the Osaka Group.

Thus, *M. biwaensis* and *M. simplex* can be distinguished even in specimens of the genus *Monactinus* extracted from Middle Pleistocene consolidated sediments.

The establishment of the Lake Biwa–Yodo River System

Although there is only one example of molecular phylogenetic evidence for plankton, *Pediastrum*, identifiable as *Monactinus biwaensis* based on morphological characteristics measured by the side ratio of marginal cells outside of Japan, has been recorded extensively in freshwater habitats ranging from temperate to tropical regions (Khaw et al. 2020). Furthermore, as stated in the Guidebook provided by the Institute of Biology of Yokkaichi University, *M. biwaensis* is now reported from various locations throughout Japan. From this perspective, it can no longer be considered an endemic species to the Lake Biwa–Yodo

River system. However, the occurrence of *M. biwaensis* in the Karato Formation and Sahama Mud Member of the Hamamatsu Formation, which do not belong to the Lake Biwa–Yodo River system, indicates that this species inhabited waters outside the Paleo (ancient) Lake Biwa–Yodo River system at least by the Early-Middle Pleistocene. This strongly suggests that its presence in waters outside the modern Lake Biwa–Yodo River system is not solely due to human factors but is also highly likely to be influenced by geo-historical factors.

Conclusion

1. *Monactinus biwaensis*, including *Pediastrum simplex* var. *biwaense* (sic), occurred in not only the Lake Biwa–Yodo River system regions but various other locations in Japan and the world.
2. Occurrence of fossil *Monactinus biwaensis* was recorded in Plio-Pleistocene sediments of the Karato Formation and the Sahama Mud Member of the Hamamatsu Formation which outcropped outside of the present Lake Biwa–Yodo River drainage system.
3. *Monactinus biwaensis* is not an endemic species for the Lake Biwa–Yodo River system on the basis of data from recent geographical and stratigraphical occurrences.

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Contribution of authors

KM conceptualized this study, investigated of material, wrote paper; MK did molecular analysis, wrote paper partly, MH did palynological analysis of materials collected from the Karato Formation, TN sampled from the Karato Formation, M.L.W.T, AI, KI, TN sampled at Lake Biwa.

Repository of materials

SeedBank 25000-1 strain is kept in SeedBank Co. Ltd., and Palynological mount slides are deposited in the Alps Technical Research Laboratory.

References

Baykal T, Acikgöz I, Yildiz K, Bekleyen A (2004) A study on Algae in Devegecifi Dam Lake. Turkish J Bot 28: 457–472.

Buchheim M, Buchheim J, Carlson T, Braband A, Hepperle D, Krienitz L, Wolf M, Hegewald E (2005) Phylogeny of the Hydrodictyaceae (Chlorophyceae): Inferences from rDNA data. J Phycol 41: 1039–1054. https://doi.org/10.1111/j.1529-8817.2005.00129.x

El Noamani ZM, Saleh AI (2018) Cretaceous algal palynomorphs from northeast Sinai, Egypt: systematics and paleoenvironmental implications. Egyptian J Bot 58: 63–72. https://doi.org/10.21608/ejbo.2017.1838.1127

Fukushima H (1956) A list of Japanese freshwater algae including the marine species of blue-green algae and fossil diatoms 2. J Yokohama Municipal Univ Ser C 13(46): 1–13. (in Japanese)

González C (2009) Catálogo de las algas y cianoprocariotas dulciacuícolas de Cuba. Universo Sur, Universidad Cienfuegos, Cienfuegos, Cuba, pp. 147.

Guiry MD, Guiry GME (2016) *AlgaeBase*. World-wide electronic publication, National University of Ireland, Galway. http://www.algaebase.org; searched on 10 November 2025.

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic acids symp. Ser. 41: 95–98.

Harada K (1984) Study of dinoflagellates from the submarine strata at the Kansai International Airport in Osaka Bay off Senshu, Central Japan. In: Kansai Kokusai Kuko Jiban Chisitsu Chosa) (ed Nakaseko K). Inst Disast Sci Japan. pp. 77–90. (in Japanese)

Imazu T (1973) Reservoir floating plants in Banshu Plain- Reservoir as an environment for floating plants. In: Current Status of Nature in Hyogo Prefecture (ed Hyogo Nature Conservation Association) Hyogo Pref Life Depart Nature Div Hyogo Jpn, pp. 57–73. (in Japanese)

Imura H (2013) Development of Ayu fry Supply and Ayu culture industry in Shiga Prefecture. Chiiki-Gyogyo-Kenkyu, 53: 25–45. (in Japanese)

Ishizaki S, Akazawa T, Yatsunami M (2001) Phytoplankton and benthos of the detention pond originated from Isahaya-bay land reclamation (2001). Bull Nagasaki Pref Publ Heal Pollut Res Inst 47: 89–91. (in Japanese)

Ishizaki S, Yoshihara N, Yatsunami M (2002) Phytoplankton and benthos of the detention pond originated from Isahaya-bay land reclamation (2002). Bull Nagasaki Pref Publ Heal Pollut Res Inst 48: 102–105. (in Japanese)

Japan Water Agency Kansai Yoshinogawa Branch Yodogawa Headquarters Hitokura Dam Management Office (2020) Hitokura Dam periodic Report, Japan Water Agency. Organisms, pp. 268. (in Japanese)

Jena M & Adhikary, S (2011) Algal diversity of Loktak Lake, Manipur. Nelumbo 53: 21–48.

Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol biol evol 30: 772–780.

Kayama M, Imura A, Tanimura WLM, Ishii K, Matsuoka K (2025) *Monactinus biwaensis*, a new combination for *Pediastrum biwae* Negoro (Chlorophyceae)—characterization of its morphology, molecular phylogeny, and asexual reproduction. Bull Osaka Mus Nat Hist No. 79: 27–47.

Khaw YS, Khong NMH, Shaharuddin NA, Yusoff F Md (2020) A simple 18S rDNA approach for the identification of cultured eukaryotic microalgae with an emphasis on primers. J Microbiol Meth 172: 105890.

Kim Y-J, Kim H-S (2012) Chlorophyta: Chlorophyceae: Chlorococcales I: Micractiniaceae, Botryococcaceae, Characiaceae, Hydrodictyaceae (Freshwater Green Algae). Nat Inst Biol Res Algal Flora Korea 6(2): 1–117.

Loaiza-Restano AM (2013) Família Hydrodictyaceae (Sphaeropleales, Chlorophyceae) no Etao de São Paulo: lantamnto floístico. Dissertação (Mestrado) - Inst Bot Secret Estado Meio Amb São Paulo Brazil, pp. 164.

Martinez-Goss MR, Arguelles EDLR, Nacorda JOO (2016) Some *Pediastrum* species (Chlorophyceae) from Laguna de Bay (Philippines) and its vicinities. Philip Scient 53: 31–51.

Matsuoka K (1972) Plant microfossils from the Saho Formation with a special reference with dinoflagellates, Yamato-Chigaku 18: 24–28. (in Japanese)

Matsuoka K (1976) Paleoenvironmental study of the Saho and the Saidaji Formations from a view point of palynology. Bull Mizunami Fossil Mus No. 3: 99–118.

Matsuoka K, Hase K (1977) Fossil *Pediastrum* from the Pleistocene Hamamatsu Formation around Lake Hamana, central Japan. Trans Proc Palaeontol Soc Jpn NS. 104: 432–441

McManus HA, Lewis LA (2005) Molecular phylogenetics, morphological variation and colony-form evolution in the family Hydrodictyaceae (Sphaeropleales, Chlorophyta). Phycol 44(6): 582–595. https://doi.org/10.2216/0031-8884(2005)44[582:MPMVAC]2.0.CO;2.

McManus HA, Lewis LA, Schuly ET (2011) Distinguishing multiple lineages of *Pediastrum duplex* with morphometrics and a proposal for *Lacunastrum* gen. nov. J Phycol 47(1): 123–130. https://doi.org/10.1111/j.1529-8817.2010.00940.x

Mizuno T (1952) Limnological studies of ponds on the land in the northern suburbs of Osaka. Jpn J Limnol. (Abstract in Proceedings of the 1951 annual meeting of the Japanese Society of Limnology in Nagoya) 16: 34. (in Japanese)

Mizuno K (2018) Consideration of the formation age of the Beppu Wan - Iyo Nada as an inland sea. Chikyu Monthly/Special No. 69: 48–54. (in Japanese)

Naidu RBV, Raju PC, Ranganayakulu GS (2018) Taxonomic Diversity of *Pediastrum simplex* in Andhra Pradesh. Bull Pure Appl Sci 37 B: 84–88.

Nakaseko K, Nishiwaki N (1984) Correlation of the submarine stratigraphy of Osaka Bay off Sensyu, central Japan with the global climatic change curve of the Quaternary. In: Kansai Kokusai Kuko Jiban Chisitsu Chosa (ed Nakaseko K) Inst Disast Sci Jpn, pp. 173–176. (in Japanese)

Naselli-Flores N, Barone R (2011) Invited Review—Fight on Plankton! or Phytoplankton Shape and Size as Adaptive Tools to Get Ahead in the Struggle for Life. Cryptog Algol 32(2): 157–204. https://doi.org/10.7872/crya.v32.iss2.2011.157.

Nasu T (1971) Note on *Pediastrum* (green algae) and pollen from the Ko-Biwako group in Kinki District, Japan. The Quaternary 16: 88–92. (in Japanese)

Naya T, Mizuno K, Hongo M, Haneda Y, Horiuchi Y (2024) Depositional age of the Pleistocene Karato Formation in Himeshima Ialand, Oita Prefecture and its stratigraphic relationship with the Himeshima Volcanic Group. Abstract of the 131st Annual Meet Geol Soc Jpn T15-O-21. (in Japanese)

Negoro K (1954) *Pediastrum Biwae* spec. nov., eine neue planktische Grunalge aus dem Biwasee. Acta Phytotax Geobot 15: 135–138.

Negoro K (1959) Some noticeable plankton algae of Lake Biwa and its effluent algae indicator of a water system. Mem Coll Sci Univ Kyoto Ser. B 26: 311–314.

Nguyen L-T, Schmidt HA, von Haesele B, Quang Minh B-Q (2015) IQ-TREE: A Fast and Effective Stochastic Algorithm for Estimating Maximum-Likelihood Phylogenies. Mol Biol Evol 32: 268–274. https://doi.org/10.1093/molbev/msu300

Prasertsin T, Pekkoh J, Pathomaree W, Peerapornpisal Y (2014) Diversity, new and rare taxa of *Pediastrum* spp. in some freshwater resources in Thailand. Chiang Mai J Sci 41(5.1): 1065–1076.

Satoguchi Y (2017) Consideration of paleo-water system changes based on geological historyaround paleo-lake Biwa basin, central Japan. J Fossil Res 50: 60–70. (in Japanese)

Singh HP, Khanna AK (1976) Some fossil species of *Pediastrum* and their palaeoecological significance in the Subathu Formation of Himachal Pradesh. J Palaeosci 25: 466–467.

Sugiyama Y (1991) Right-lateral strike-slip basins in the Second Paleo-Seto Inland Sea—A model of basin development associated with the migration of active domain of a large-scale strike-slip fault-. Kozo-Chishitu (Tect Geol), 36: 99–107. (in Japanese)

Tanaka M (2022) Illustrations of the Japanese Freshwater Plankton. 2nd edition. The Univ. Nagoya Press, pp. 747. (in Japanese)

Wilson LR, Hoffmeister WS (1953) Four new species of fossil *Pediastrum*. Am J Sci 251: 753–760. https://doi.org/10.2475/ajs.251.10.753.

Xiang L, Huang X, Huang C, Chen X, Wang H, Chen J, Hu Y, Sun M, Xiao Y (2021) *Pediastrum* (Chlorophyceae) assemblages in surface lake sediments in China and western Mongolia and their environmental significance. Rev Palaeobot Palynol 289: 104396.

Yamagishi T, Hirano M (1973) Some freshwater algae from Cambodia. Cont Biol Lab Kyoto Uni 24(2): 61–85.

Zamaloa MC, Tell G (2005) The fossil record of freshwater micro-algae *Pediastrum* Meyen (Chlorophyceae) in southern South America. J Paleolimnol 34: 433–444. https://doi.org/10.1007/s10933-005-5804-8