Morphological evolution of *Stephanodiscus* (Bacillariophyta) in Lake Biwa from a 300 ka fossil record

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ABSTRACT: A series of intermittent changes in *Stephanodiscus* (Bacillariophyta) valve morphology was observed in sediment core materials collected from Lake Biwa, Japan. A constant sedimentation rate reveals a continuous but punctuated pattern of morphological evolution. Morphological parameters, including maximum diameter and fascicle number v. diameter ratio (fascicle density), showed long periods of stasis and less than 10 000-year directional changes. Specifically, directional decreases in fascicle density occurred at 200–190 and 160–150 ka, and increases in maximum diameter occurred at 130–120 ka. According to a morphology-based species definition, a non-branching phylogenetic lineage originating from a cosmopolitan ancestor can be presumed from a morphology-based cladistic analysis and the biostratigraphic record. Evolutionary modes were selected from directional change, random walk, stasis and combined models by a statistical test, paleoTS, to clarify that the sequential changes are composed of relatively long stasis modes and short directional change modes. Global climate change was probably an influence during the early stages of the history of Lake Biwa.

KEYWORDS: *diatoms; evolutionary rate; Lake Biwa; morphological evolution; phylogeny*

SUPPLEMENTARY MATERIAL: Time-series data to discriminate the tempo and mode of morphological evolution are available at http://www.geolsoc.org.uk/SUP18825

INTRODUCTION

The fossil record is a good resource with which to demonstrate the tempo and mode of evolution. Biological laboratory studies and fieldwork demonstrate observable changes in genetic composition and the small modifications of evolutionary phenomena. However, many aspects of evolution, including large-scale modification of morphology and speciation, occur over long periods of geological time. Only the fossil record can reveal the tempo and modes of phenotypic changes as preserved in the hard parts of organisms. Speciation is a principal topic in evolutionary history and an essential driver of biological diversity both in the present and the past.

The outstanding work of Eldredge & Gould (1972) encouraged discussions of speciation in palaeontological studies. However, obtaining empirical results from the fossil record is not as simple as Eldredge & Gould expected. Various tempos and modes of speciation have been reported in the 20 years following (Erwin & Anstey, 1995), which have inspired questions for future research. For example, whether a particular tempo and mode prevail in a particular environment, time, life history strategy or geographical condition (Erwin & Anstey, 1995) is, as yet, poorly understood.

Evolutionary tempo (i.e. the rate of evolution) as re-analysed by statistical models has revealed few real cases of punctuation (Hunt, 2008*a*). This is partly due to the existence of directional change, which resembles a random walk in the decreased time resolution of the fossil record (Roopnarine *et al.*, 1999). The unambiguous tempo of evolution should be examined with good time resolution, moving beyond the gradualism/punctuated equilibrium dichotomy. Of the hundreds of fossil sequences published, few are of long duration and highly resolved (Hopkins & Lidgard, 2012). Studies of Late Neogene *Globorotalia* foraminifera (Malmgren *et al.*, 1983) and Holocene lacustrine diatoms (Theriot, 1992) may permit such length and time resolution, although the material restricts the former study to a coarser time resolution and the latter study to a short time range. The current study examines the tempo and mode of morphological evolution in planktonic diatoms from Lake Biwa in the highest time resolution and over a sufficiently long time range.

Sediments deposited on the bottom of lakes are a continuous record of the past. In general, the time resolution of lacustrine sediment is higher than that of marine sediments because lakes have abundant sedimentary input. The tempo of evolution should be examined with such sediment providing high resolution. Diatoms comprise a major group of phytoplankton in Late Neogene lakes, and their siliceous frustules preserve well in the sediments (Barron, 1987). The evolutionary history of diatoms, at least from a morphological aspect, should be precisely recorded in the sediments. This study presents a time-series documenting the tempo of morphological evolution based on the continuous record of a sediment core recovered from Lake Biwa. The lake is a so-called ancient lake, which has developed over more than 400 000 years a unique ecosystem that includes endemic species (Nishino & Watanabe, 2000). The chronology and sedimentation rate are estimated by 13 dated volcanic tephras intercalated in the core.

The mode of speciation is more difficult to distinguish than modes of morphological evolution. The number of species varies according to different definitions of the species category, and the number of speciation events depends on this definition. The mode of speciation (i.e. the degree of branching) could change as larger numbers of species are recognized. More than 20 species concepts have been published to date (Wheeler & Meier, 2000), but none can deal equally with every taxon of extant and extinct species. For convenience, this study adopts a morphology-based definition and concept of species to discuss modes of morphological evolution and the phylogeny of diatom fossils.

Large lakes with a long history often have high biodiversity. For example, plenty of endemic species of cichlid fish have been identified in the East African Great Lakes. One of these, Lake Victoria, experienced almost complete desiccation during the last deglaciation period (Johnson *et al.*, 1996). A strikingly rapid diversification of cichlids could occur within 15 000 years following such an event, in which case the resulting clade is interpreted as a superflock (Verheyen *et al.*, 2004). In contrast, extant planktonic diatoms in the oldest lake, Lake Baikal, are not diverse (Edlund *et al.*, 1996). A few species have always dominated the community, and these are often endemic species distributed in the lake or surrounding area (Khursevich *et al.*, 2004). A less branching pattern of evolution is inferred in this case, with an over 10 000-year-long stability in the occurrence of the endemic species. The tempo and mode discussed in this article show detailed stratigraphic changes in diatom morphology. Our observations may represent the development of endemism and low species diversity as well as explain the origin of diatom flora in ancient lakes.

MATERIALS AND METHODS

Lake Biwa is the most recent of a series of tectonic lakes in central Japan that formed and filled with sediments starting in the Late Pliocene (Kawabe, 1981; Yokoyama, 1984). Since the 1970s, several cores have been taken from this lake, and multiproxy analyses of over 200 m of continuous clay sediments have been used to reconstruct past climates from the Middle–Late Pleistocene to the present. Tephrochronology, palaeomagnetism and fission track dates estimate a 430 000-year history of continuous sediment deposition (Meyers *et al.*, 1993). In addition to the sedimentary records, the present biota of Lake Biwa has been well documented, with a rich species diversity including endemic fish and gastropods (Nishino & Watanabe, 2000) and algal species (Tuji & Hoki, 2001).

Materials for this investigation were subsampled from core samples referred to as the '200 m core' taken in 1971 (Mori, 1974, 1975; Mori & Horie, 1984). The core site of the 200 m core had a *c.* 65.2 m water depth and was located at a ridge separating sub-basins in northern Lake Biwa (Fig. 1). Material recovered from this site represented a 197.2 m continuous sediment record (Horie, 1972). More than 40 volcanic tephra layers were recognized in this sediment record, 13 of which were correlated with widespread and dated tephras from the Japanese Islands and the surrounding area. This demonstrated a reliable correlation between the 200 m core and the other long cores, the Takashima-oki core and the 1400 m core (Yoshikawa & Inouchi, 1991, 1993). The oldest identified tephra layer in the 200 m core (181.3 m) is the Hatta volcanic ash (Machida *et al.*, 1991), which was originally reported from a marine section (Ma 10) of the Osaka Group

deposited at *c.* 300 ka (Yoshikawa & Inouchi, 1993). We used the revised ages of the tephra layers by Machida & Arai (2003) for our chronology, which permits interpolated and extrapolated ages for each stratigraphic horizon (Fig. 2).

Over 700 subsamples, 5 cm thick and *c.* 25 cm intervals in depth from the 200 m core, were preserved for diatom analysis. The dried sediments in glass bottles were supplied by one of the authors, Shinobu Mori, and are deposited in the National Museum of Nature and Science, Japan. We chose samples of 5 cm thick sediment representing a 100-year average diatom flora, and our sampling interval was 50 cm corresponding to a *c.* 1000-year resolution, based on the accumulation rate and the bioturbation of the sediment. Dried materials were weighed and boiled in 15% hydrogen peroxide (H_2O_2). This H_2O_2 diatom slurry was rinsed four times with distilled water and was bottled as 15 ml suspensions. Methods for light microscopy (LM) and scanning electron microscope (SEM) observations follow Kato *et al.* (2003). An Olympus BX-51 LM equipped with differential interference contrast optics and a JEOL JSM 5310 SEM were used for all observations.

Diagnostic morphological features useful for identifying discoidal *Stephanodiscus* species were used. These features included the numbers of fascicles and spines, and the external morphology of the mantle strutted processes. Many of these morphological characters are dependent to some degree on valve diameter, and a scatterplot of each parameter v. diameter provided complete separation of the taxa (Theriot & Stoermer, 1984). We report the fascicle number v. valve diameter ratio (i.e. fascicle density) to represent areolation patterns in valve morphology. Valve diameters for 50 randomly selected specimens from each sedimentary interval were measured at 1000× magnification in the LM using an optical micrometer with 0.5 µm precision. The numbers of fascicles on each valve were enumerated for the same specimens that were measured for diameter to calculate fascicle density. The spine patterns on each studied valve were described by LM observation as regularly or irregularly arranged. The valve diameter of every initial valve occurring on each of the LM slides was measured. The external structures of the mantle strutted processes of 20 specimens were observed with SEM from the section showing rapid changes in the fascicle density, from 93.12 to 131.18 m depth.

A cladistic analysis was conducted based on Theriot's (1992) data matrix to test the evolutionary phylogeny of Lake Biwa *Stephanodiscus* taxa inferred by the biostratigraphy. The only modification of the matrix was the addition of taxa from Lake Biwa and a single character, the presence or absence of an external mantle strutted process tube (Appendix A; Table 1). The cladogram was rooted by an outgroup, *Cyclostephanos invisitatus* (Hohn & Hellermann, 1963; Theriot *et al.*, 1987) (Table 1). The branch and bound procedure in PAUP version 4.0b10 (Swofford, 1998) was used to determine the most parsimonious cladograms. Character optimizations were explored using MacClade, version 4.08 (Maddison & Maddison, 2005).

Statistical approaches to discriminate the tempo and mode of morphological evolution were performed using the paleoTS version 0.4-4 R package (Hunt, 2006, 2008*a*, *b*; Hunt *et al.*, 2010). This software package facilitates analysis of palaeontological sequences of trait values from an evolving lineage. Evolutionary models including general random walk (GRW, i.e. directional evolution), unbiased random walk (URW), stasis (Stasis), unsampled
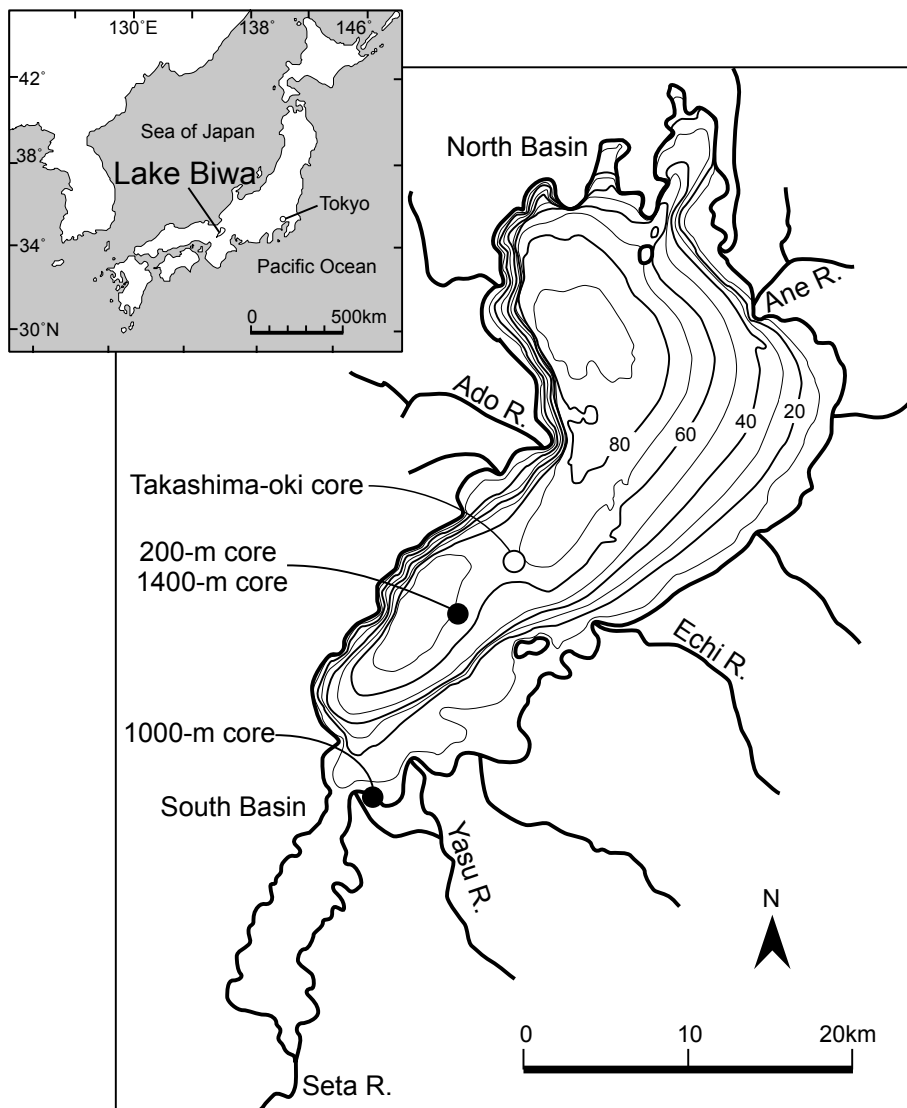
Fig. 1. Bathymetric map showing coring site of 200 m core and the other long cores taken from Lake Biwa. Localities of 200 m core from Horie (1972), of 1400 m core from Miyoshi *et al*. (1999), of 1000 m core from Takemura *et al*. (1976) and Takashima-oki core from Yoshikawa & Inouchi (1991, 1993).

punctuation (Punc-1) and sampled punctuation (SGS, stasis-GRW-stasis) were applied to the sequences of fascicle density and diameter of Lake Biwa *Stephanodiscus*, consisting of 229 values from 229 horizons. The minimum lengths of the segments in the Punc-1 and SGS models were 8 for both analyses of diameter and fascicle density. Subsetting series of the sequence in a sliding window including 10, 20 or 30 values of fascicle densities and diameters ($n=10$, 20 or 30) from 10, 20 or 30 horizons was provided to determine temporal evolutionary mode from the complex pattern of the sequences. Five (GRW, URW, Stasis, Punc-1 and SGS) models were compared to determine the temporal evolutionary mode (Appendix B). Best-fit models were chosen by the Akaike information criterion corrected for small sample size (AICc).
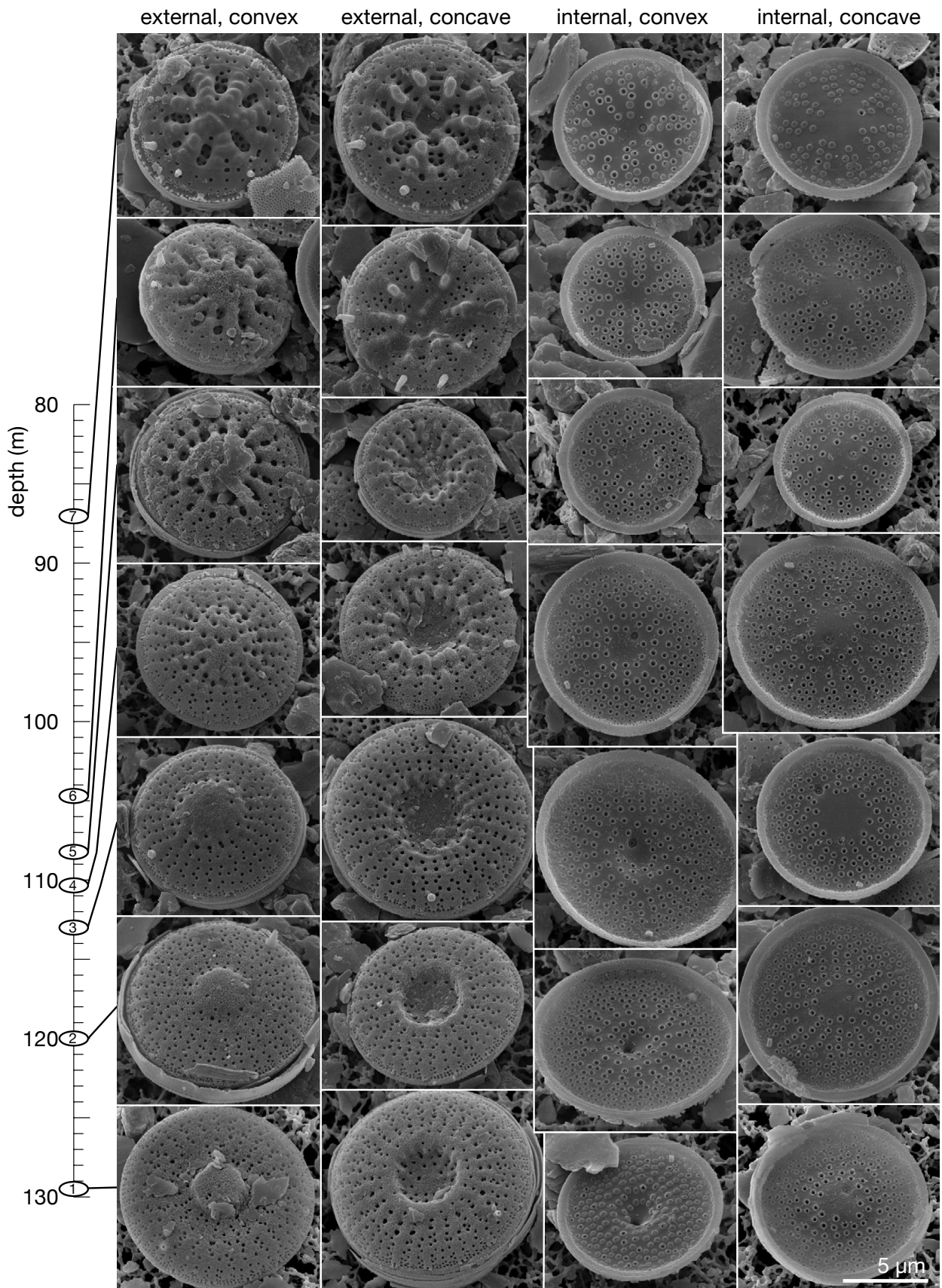
RESULTS
Stratigraphic changes in the diatom flora
In general, the concentration of valves of all diatoms is high during warm periods corresponding to marine isotope stages (MISs)

1, 5 and 7, whereas it is low in cold periods like MISs 2, 6 and 8 (Fig. 3). *Aulacoseira* species, including *A. nipponica* and related taxa (see Appendix C), contribute greatly to the rapid increases and decreases in the valve concentration of all diatoms. In contrast, *Stephanodiscus* species, including the *S. suzukii* group, *S. vestibulis, S.* cf. *niagarae* and *S.* cf. *oregonicus*, seem to respond slowly to climatic changes. Stratigraphic changes in the valve concentration of *Stephanodiscus* specimens that more or less resemble *S. suzukii* or *S. vestibulis* (Pl. 1) are shown together with those of the *S. suzukii* group and *S. vestibulis* (Fig. 3). These taxa increase above the Ata-Th tephra (240 ka), reaching the highest value at the horizon near the Aso-3 tephra (120–135 ka). They decrease in the section corresponding to the last glacial period and slightly increase during the last 10 000 years.

Stratigraphic changes in the valve morphology of the *Stephanodiscus suzukii* group
Stephanodiscus specimens with morphological combinations identical to extant *S. suzukii* and some with modifications were
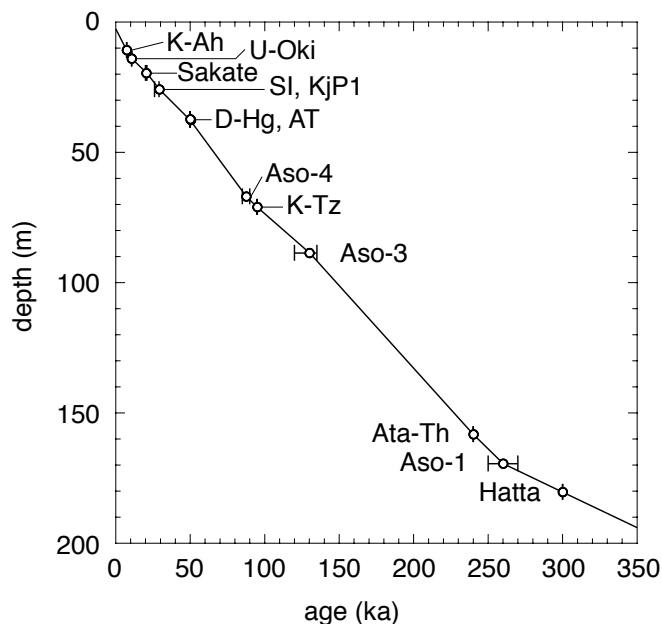
external, convex external, concave internal, convex internal, concave

depth (m)

80

90

100

110

120

130

7

6

5

4

3

2

1

5 μm

Fig. 2. Chronological model of 200 m core based on ages of 13 volcanic tephra layers. The revised ages of tephra layers by Machida & Arai (2003) are utilized for our chronology and allow interpolated and extrapolated ages for each stratigraphic horizon.

observed (Pl. 1). The valve morphology became more modified when the specimen was taken from deeper sections (Pl. 1). Qualitatively, fascicles on the valve face were thinner in older sections and became broader in younger sections, with the changes occurring intermittently. Valve morphology shows little change over several tens of thousands of years, whereas it changes quickly within several thousand years. Numerical measurement supports the degree of modifications (Fig. 4). The average fascicle density of 50 valves of *Stephanodiscus* was between 2.5 and 3.5 in the section below 130 m depth, quickly decreasing to 2.0 and slightly increasing to 3.0 in the second plateau. Fascicle density decreased significantly between 110 and 105 m depth, creating another plateau at 1.0–1.5, and became stable at *c.* 1.0 above 85 m depth. Based on morphological stasis and unique combinations of morphological features, four morphology-based species are defined: *S. suzukii, S. praesuzukii, S. umbilicus* and *S.* cf. *vestibulis*. Morphological features of each of the species are described in detail in Appendix C.

A cladistic analysis was performed to test the phylogenetic hypothesis that the four Lake Biwa taxa discussed here represent a single evolutionary group. This analysis produced three equally parsimonious cladograms, and the Lake Biwa taxa were fully resolved. The strict consensus tree is shown in Figure 5. The original intent

of this matrix was to evaluate morphological features exhibited by members of the *Stephanodiscus niagarae* species complex. Our utilization of the data matrix stretches its original intent, but allows resolution of the group being considered here (Fig. 5) and demonstrates that the Lake Biwa taxa represent a clade independent from other taxa considered by Theriot (1992).

The cladogram corroborates the evolutionary history inferred from the morphology-based biostratigraphy from Lake Biwa. The biostratigraphic occurrence of *Stephanodiscus* species in Lake Biwa suggests anagenetic change (Smith, 1994) without bifurcation and does not show cladogenetic change that increased species diversity. The morphology-based biostratigraphy in the present study suggests that morphological change occurred in the entire population. In the phylogenetic tree, immediate shifts from the ancestor to the anagenetic descendant are presumed.

DISCUSSION

Uneven tempo and mode of morphological evolution were detected using the paleoTS statistical test. Best-fit models of whole time-series data are shown in Table 2. The Stasis-Directional change-Stasis (SGS) model was selected for both the diameter and fascicle density traits with extremely strong support (Table 2). Shift points are 9 (223.2 ka) and 104 (113.9 ka) for diameter, and 19 (205.1 ka) and 106 (112.2 ka) for fascicle density (Table 2). Directional changes between older and younger Stasis modes extend between 223.2 and 113.9 ka for diameter and between 205.1 and 112.2 ka for fascicle density. Both directional changes include obvious changes in diameter between 130 and 120 ka and in fascicle density at around 200 ka and between 160 and 150 ka, although the length of directional change in diameter is greater.

To examine the patterns of the time-series in finer detail, subsets of the sequences ($n = 10$, 20 and 30) were analysed. When n became smaller, the Punc-1 and SGS models were rarely selected (Fig. 6) because of the AICc disadvantage for models with many parameters. Results of fascicle density show that SGS models were selected during 220–140 ka when $n = 30$ (Fig. 6a). Random walks and directional changes were the best in two sections around 200 ka and 160–150 ka when $n = 20$ (Fig. 6b) and directional changes and higher numbers of random walks were selected instead of punctuations when $n = 10$ (Fig. 6c). In contrast to fascicle density, diameter time-series resulted in an indistinct pattern. Random walks and SGSs were dominant in the 140–120 ka section; however, SGSs were selected in older and younger sections (Fig. 6e, $n = 30$). Stasis modes increased when n became smaller (Fig. 6f, g) instead of punctuations, which dominated when $n = 30$ (Fig. 6e). Random walks also increased in the $n = 10$ analysis (Fig. 6g).

Hunt (2012) discussed the inseparability of tempo and mode and stated that no rate metric works well for all traits and time-scales.

Explanation of Plate 1. SEM images of transitional forms developing the broad fascicles and raised costae and depressed areolated area with decreasing fascicle density. Specimens showing external and internal sides of convex and concave valves from seven selected horizons; 1, 319d (129.44 m); 2, 295b (120.08 m); 3, 277a (113.16 m); 4, 267a (110.40 m); 5, 261a (108.39 m); 6, 251a (104.70 m); 7, 203a (87.11 m). External views of convex valves are shown on the left, external views of concave valves on the left middle, internal views of convex valves on the right middle and internal views of concave valves on the right side for each horizon. Scale bar on the bottom right for every image.

Table 1. Data matrix for investigated *Stephanodiscus* taxa and an outgroup.

Taxa	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	Source*
hantzschii	0	0	0	0	0	0	0	0	1	1	0	0	0	0	2	0	?	1	1	0	0	0	0	0	1	2
minutus	0	0	0	1	0	0	0	0	1	1	0	0	0	1	0	0	0	1	1	0	0	0	0	0	0	2
vestibulis	0	0	0	1	0	0	1	0	1	1	0	0	0	1	0	0	0	1	0	0	1	1	0	0	0	2
carconensis	0	0	0	0	0	0	0	1	1	0	0	1	1	3	2	0	?	1	1	0	0	0	1	2	1	2
subtransilvanicus African fossil	0	0	0	0	0	0	0	1	1	1	0	0	0	1	0	0	?	1	1	0	0	0	1	2	0	2
subtransilvanicus Laurentian Great Lakes	0	0	0	0	0	0	0	1	1	0	0	0	0	1	0	0	?	0	1	0	0	0	1	2	1	2
agassizensis	1	0	1	2	0	0	0	0	1	1	0	0	0	1	0	0	0	1	0	0	0	0	0	1	0	2
alpinus	1	0	1	0	0	0	0	0	1	1	0	0	0	1	0	0	0	1	0	0	0	0	0	1	0	2
neoastraea	1	0	2	0	1	0	0	0	1	0	0	0	0	1	0	0	?	1	1	0	0	0	0	2	0	2
galileensis	1	0	2	3	1	0	0	0	1	0	0	0	0	1	0	1	1	1	1	0	0	0	0	2	0	2
rotula	2	1	2	3	1	0	0	0	1	0	0	0	0	1	1	0	0	1	1	0	0	0	0	2	1	2
niagarae	2	1	3	2	1	0	0	0	1	0	0	0	1	1	1	1	0	1	1	0	0	0	0	2	1	2
reimerii	2	1	3	2	1	0	0	0	1	0	0	0	1	1	1	0	0	1	1	1	0	0	0	2	1	2
yellowstonensis	2	1	4	2	1	1	1	0	1	0	0	0	1	1	1	0	0	1	1	0	0	0	0	2	1	2
superiorensis	2	1	3	1	1	0	0	0	1	0	1	0	1	2	1	0	0	1	1	0	0	0	0	2	1	2
cf. *vestibulis* L. Biwa fossil	0	0	1	1	0	0	1	0	1	1	0	0	0	3	0	0	1	1	0	0	1	1	0	0	0	1
umbilicatus L. Biwa fossil	0	0	?	1	0	?	1	0	?	?	0	0	0	3	0	0	1	1	0	0	1	1	0	0	1	1
praesuzukii L. Biwa fossil	0	0	1	1	0	0	1	1	1	1	0	1	0	3	2	0	1	1	0	0	1	0	1	0	1	1
suzukii L. Biwa	?	0	1	1	0	0	1	1	1	1	0	1	0	3	2	0	1	1	0	0	1	0	1	1	1	1
Cyclostephanos invisitatus (outgroup)	0	0	0	1	1	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0

Character set (Character 0–23) and fifteen terminal taxa were imported from Theriot (1992). An original character (Character 24) for the second analysis, four Lake Biwa taxa and *Cyclostephanos invisitatus* as an outgroup, were added.

? = character not applicable.

*Numbers represent sources of observations: 1, this study; 2, Theriot (1992).

The rate of evolution, however, may be compared by each expected squared divergence (ESD), a measure of the predicted magnitude of change by a model over a specified interval of time. Figure 6 shows ESDs from the best-supported models in 1000-year spans, which correspond to the average interval of the morphological data. In the fascicle density time-series, ESDs were higher in two sections (210–200 and 170–150 ka) (Fig. 6d), when directional changes and random walks were preferred. ESD values in the diameter time-series (Fig. 6h) were 10 or 100 fold larger than those for fascicle density (Fig. 6d). ESDs for diameter slightly increase in sections younger than 100 ka, but the trend is weak (Fig. 6h).

Disagreement in the paleoTS results between fascicle density and diameter is common in morphological evolution. Hopkins & Lidgard (2012) concluded that different evolutionary modes were frequently detected in different traits of the same lineage. A part of the disagreement in Lake Biwa *Stephanodiscus* species may be explained by small variations in fascicle density within a population, but large variations in diameter, which undergoes much greater variation in the diatom life cycle (Round *et al.*, 1990). Life cycle and cell size are also easily affected by the environmental setting (Potapova & Snoeijs, 1997).

In general, these analyses demonstrated that Lake Biwa *Stephanodiscus* species experienced distinct evolutionary modes intermittently. Stasis modes lasted for a longer time, whereas a GRW mode suggesting directional evolution tended to end in a short time period (Fig. 6). This pattern may be applicable as an example of punctuated equilibrium as suggested by Eldredge & Gould (1972), but it did not show a branching pattern. Our fossil evidence from Lake Biwa also cannot determine if one species that originated from part of an ancestral population survived while the remainder became extinct immediately or if the entire population of the ancestor turned into the descendant species. The former speculation is probable in this case because it is not uncommon that a single species or a few dominant species are dominant in a temporal planktonic diatom community (Edlund, 2006). The descendant and the ancestral species cannot share their ecological position if they require a similar environment.

Climate change influenced the rapid directional changes partly because the morphological changes tended to occur in transitional periods from warm to cold climate or vice versa. The earliest change corresponds to the MIS 7/6 transition, the second to that from a warm to a cold episode in MIS 6 and the latest to MIS 6/5. Synchronous changes in diatom valve morphology and environmental factors during a climatic transition (from the last glacial to Holocene period) were also reported by Theriot *et al.* (2006). In Lake Biwa, however, the morphological changes did not occur in the last climate transition, and stable phases dominate the latter half of the lake history, including the last glacial to the Holocene period. A deep open-water environment maintained in a
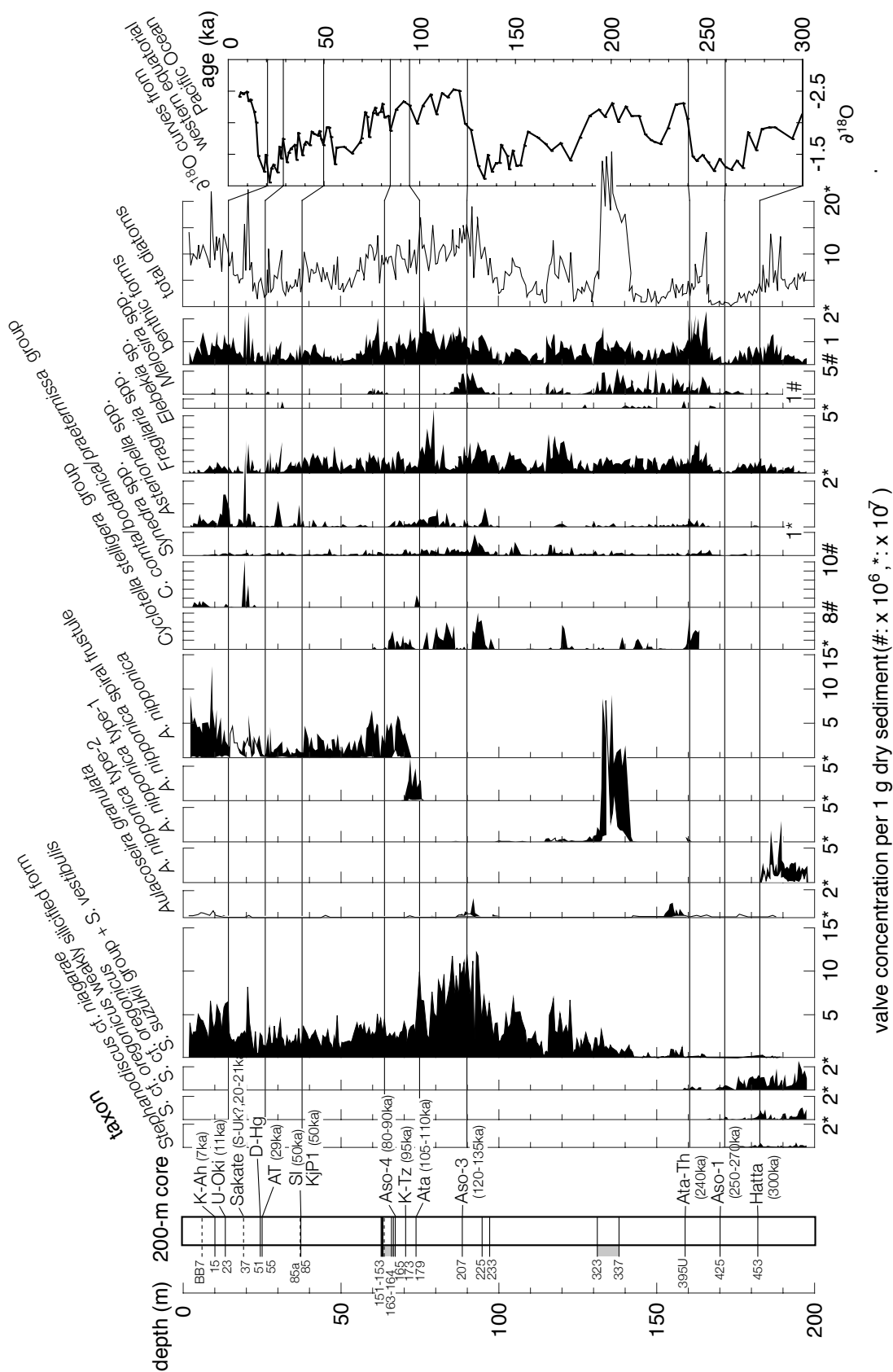
Fig. 3. Stratigraphic distribution of diatom flora from 200 m core. Identified volcanic ash layers are shown on the columnar section with sample numbers and estimated ages. For *Aulacoseira* species, concentrations of thin forms are shown in white and the thick ones in black. A 400 ka marine oxygen isotope record from De Garidel-Thoron *et al.* (2005) is shown on the right.
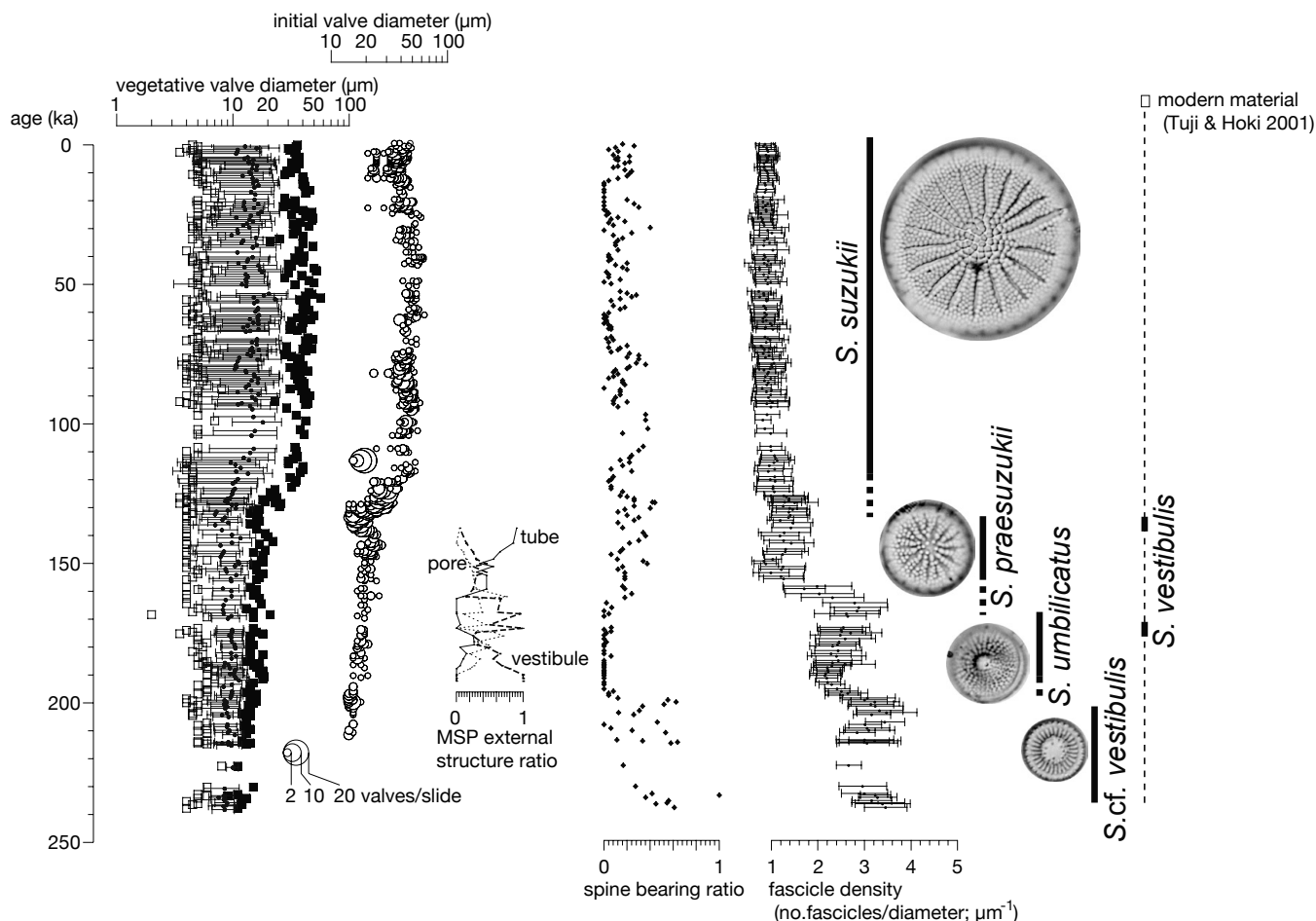
Fig. 4. Stratigraphic changes in valve morphology of *Stephanodiscus* species, which occurred monospecifically. From left to right; valve diameter, open squares (minimum), solid small circles (average) and solid squares (maximum); distribution of initial valve diameter; frequency of vestibule, pore or short tube for mantle strutted process external opening, vestibule in dashed line, pore in dotted line and tube in solid line; spine-bearing valve ratio, broken line showing the exceptionally high values intercalated by *S. vestibulis*'s occurrence; distribution of fascicle/valve diameter ratio called fascicle density in this study; schematic diagram showing biostratigraphic ranges and phylogenic hypothesis for *Stephanodiscus* cf. *vestibulis, S. umbilicatus, S. praesuzukii* and *S. suzukii* in Lake Biwa with reference to *S. vestibulis*.

temperate climate with sufficient rainfall (Nakagawa *et al*., 2008) may have provided stable environments for planktonic diatoms over the last 100 000 years.

APPENDIX A: CHARACTER DESCRIPTION FOR CLADISTICS ANALYSIS

Character set of Theriot (1992) is applicable to our cladistic analysis. We use extant characters (Characters 0–23) in the first analysis and add a new character for the second analysis to gain more resolution for Lake Biwa taxa. The character, Character 24, describes presence (1) or absence (0) of external tubes of mantle strutted processes. The data matrix for the terminal *Stephanodiscus* taxa is shown in Table 1.

APPENDIX B: TIME-SERIES DATA FOR PALEO-TS0.4-4 STATISTICAL ANALYSIS AND ITS PARAMETER OUTPUTS

The best models and the second best models with their parameters are presented in the Supplementary material tables: Table B1 ($n=30$, fascicle density), B2($n=20$, fascicle density), Table B3 ($n=30$, fascicle density), Table B4 ($n=30$, diameter), Table B5 ($n=20$, diam-

eter) and Table B6 ($n=10$, diameter). Expected squared divergences (ESDs) are calculated for the best models. Grey rows represents outputs with weak statistical supports (AICc < 0.5). Time-series data for paleo-TS 0.4-4 statistical analysis are shown in Table B7.

APPENDIX C: TAXONOMIC NOTES

Stephanodiscus cf. *vestibulis* Håkansson, Theriot & Stoermer, 1986
(Fig. C1a–d)

Remarks. *Stephanodiscus vestibulis* was originally described from North American plankton samples (Håkansson *et al*., 1986). Specimens from the Lake Biwa core were consistent with described morphometric parameters, except for a central strutted process occurring in a depression. Specifically, valve diameters between 4 and 16 μm, concave and convex valves with concentric undulations (Fig. C1a, b), radiating areolar rows banded in fascicles with one row at the valve centre and two rows at the valve face margin (Fig. C1c), 3–4 smaller areolar rows on the valve mantle (Fig. C1c), obvious hyaline interfascicles raised externally from the areolated layer (Fig. C1a), slightly raised interfascicles on the internal
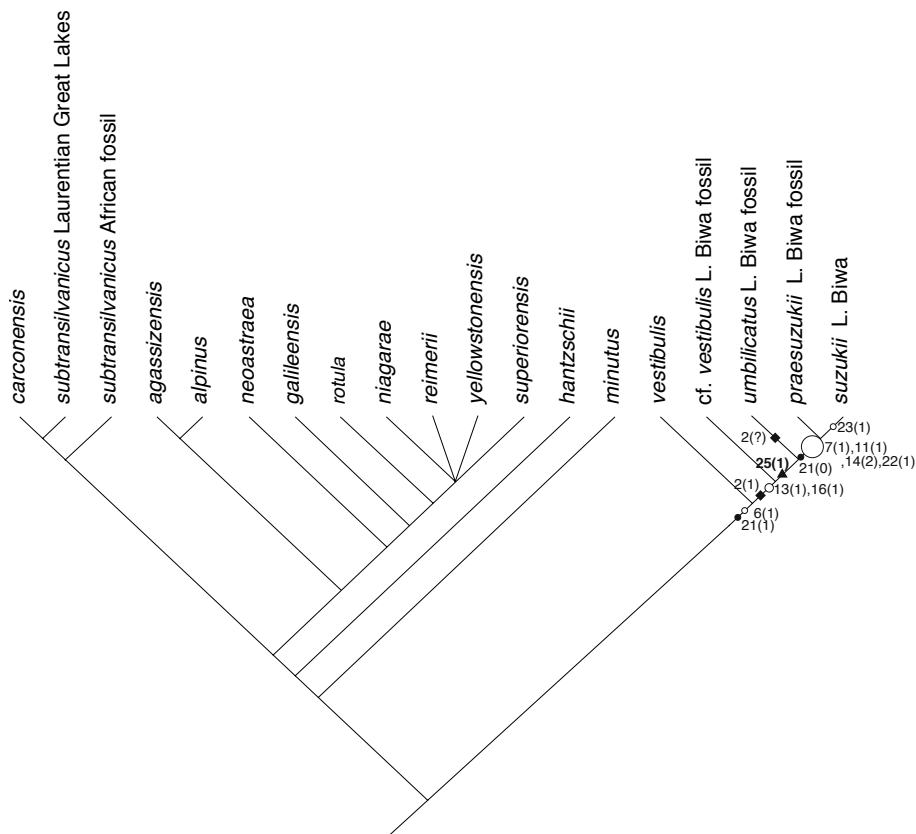
Fig. 5. Strict consensus tree of three equally parsimonious cladograms showing character state distribution in Lake Biwa taxa and *Stephanodiscus vestibulis*. Numbers on branches refer to the character number with the state number in brackets of Theriot's (1992) data matrix. They show synapomorphic character states combining sister taxa. Open circles are character states that need one step on the cladogram (large circles represent multiple characters). Solid circles and solid diamonds are those that need reversal steps in the clade. Solid triangle is the single synapomorphic character state *S. umbilicatus*, *S. praesuzukii* and *S. suzukii* to form a monophyletic clade.

Table 2. Results of paleoTS statistical test on whole time-series of diameter and fascicle density of Lake Biwa *Stephanodiscus* species.

Evolutionary model/mode	Log likelihood	AICc	Akaike weights	step mean	step variance	theta1	theta2	omega	shift1	shift2
Fascicle density										
directional (GRW)	133.23	−262.40	0	-1.21×10^{-4}	1.29×10^{-5}					
random walk (URW)	132.46	−262.91	0		1.31×10^{-5}					
Stasis	−275.07	554.19	0			1.45		0.65		
Punctuation (Stasis-Stasis)	−18.81	45.81	0			2.70	0.98	6.58×10^{-2}	63	
SGS (Stasis-directional-Stasis)	175.61	−336.72	1	-2.39×10^{-5}	2.24×10^{-5}	3.13	0.91	3.90×10^{-3}	19	106
Diameter										
directional (GRW)	−500.54	1005.13	0	-1.64×10^{-5}	3.02×10^{-3}					
random walk (URW)	−500.55	1003.11	0		3.02×10^{-3}					
Stasis	−572.33	1148.70	0			12.02		7.92		
Punctuation (Stasis-Stasis)	−454.77	917.73	0			9.34	14.16	2.30	104	
SGS (Stasis-directional-Stasis)	−436.92	888.36	1	-5.48×10^{-6}	0	8.51	14.16	3.56	9	104

valve mantle, and inconspicuously domed mantle cribra (Fig. C1c). The peculiar features, vestibule-like external openings of mantle strutted process (Fig. C1d) and short external tube of the labiate process (Fig. C1b) were also observed. The autapomorphic feature of Lake Biwa specimens was the presence of central strutted process in a circular depression (Fig. 5), or in fovi (Theriot, 1992).

Modern specimens reported from Japanese lakes (Goto *et al.*, 1998) and Lake Biwa (Tuji & Hoki, 2001) did not have fovi; small populations from the upper sections of our core also lacked this feature. We distinguish the specimens in the section 158.1–131.6 m from *S. vestibulis* because of this morphological difference. The central strutted process in fovi was commonly observed in Lake Biwa *Stephanodiscus* and the autapomorphic nature of the central strutted process occurring in fovis is sufficient for diagnosis as a phylogenetic species (Theriot, 1992).

Stephanodiscus umbilicatus Saito-Kato sp. nov.
(Fig. C2a–g)

Holotype. MPC-02657 (Micropaleontology Collection, National Museum of Nature and Science, Tokyo).
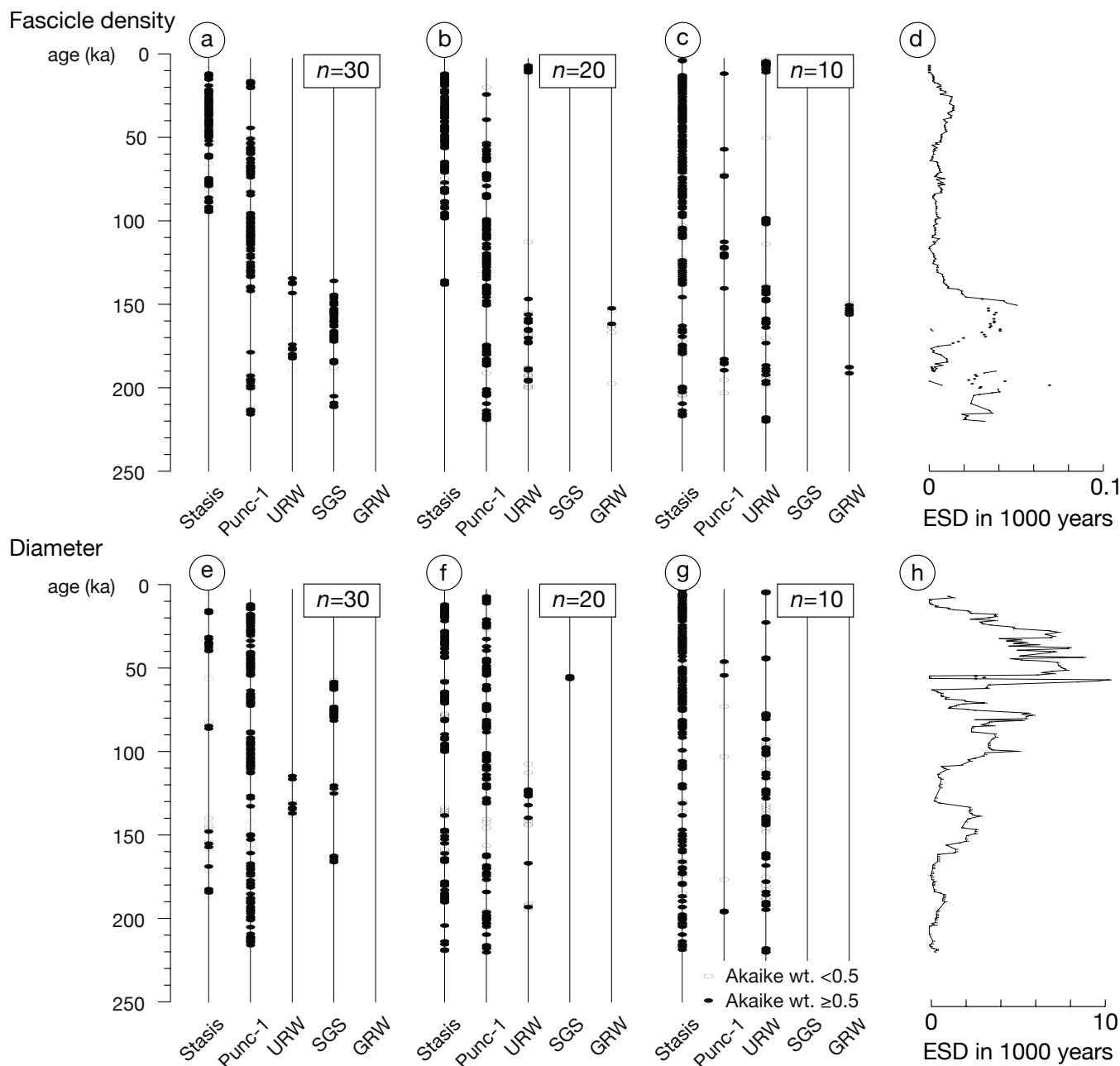
Fig. 6. Tempo and mode of morphological evolution of Lake Biwa *Stephanodiscus*, results of paleoTS statistical test. Estimated best models for each window (*n* = 10, 20 and 30) from five models, stasis, punctuation (Punc-1), random walk (URW), Stasis-directional-Stasis (SGS) and directional change (GRW) and expected squared divergences (EDSs). (**a–d**) Results of fascicle density, *n* = 10, 20, 30 and EDSs from *n* = 20; (**e–h**) those of diameter. Lines in (d) and (h) are EDSs calculated from omega of Stasis, Punc-1 and SGS modes, and dots are those from step mean and variance of URW, GRW and SGS modes.

Type locality. No. 315-b subsampled from 127.74 m depth of 200 m core, Lake Biwa, Shiga Prefecture, Japan.

Description. Valve discoid (Fig. C2a, b) ranging from 4 to 20 μm in diameter (typically smaller than 18 μm). Central area small and concave (Fig. C2c) or convex (Fig. C2d, e). Radiating areolar rows on the valve face form narrow fascicles, with one row at the valve centre and two rows at the valve margin (Fig. C2f). Smaller areolae arranged in several rows on the valve mantle (Fig. C2f). Interfascicles, i.e. costae, thin and not obvious (Fig.

C2c–e). Mantle strutted processes located on every, or every second, interfascicle, forming a ring (Fig. C2f). The external opening of mantle strutted processes is a vestibule (Fig. C2c), simple pore (Fig. C2d) or a short tube (Fig. C2e). Internally the strutted processes have three satellite pores (Fig. C2g). A single strutted process is within the valve face central concentric undulation (Fig. C2f); however, it cannot be distinguished from areolar openings in external views of the valve face. Internally it occurs in a small circular depression, referred to as fovi in Theriot (1992), and has three satellite pores. A labiate process is located slightly inside
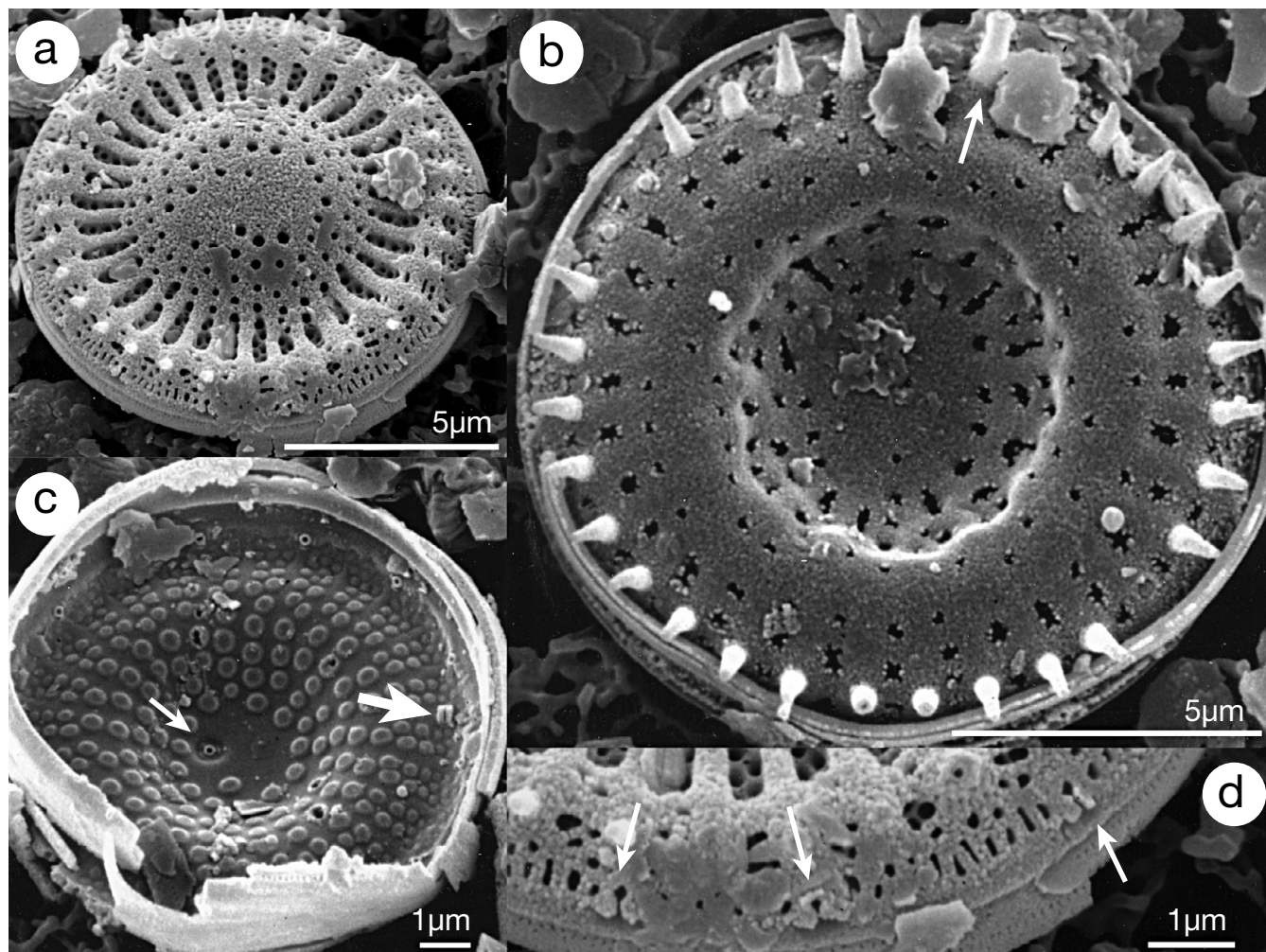
Fig. C1. SEM images of *Stephanodiscus* cf. *vestibulis* from Lake Biwa 200 m core. **(a)** External view of convex valve showing lifted interfascicles from 393-d (157.5 m depth). **(b)** Concave resting forms with occluded areolae, well-developed marginal spines and external tube of labiate process (arrow) from 329-b (133.4 m depth). **(c)** Internal view of convex valve with sessile labiate process (large arrow) and central strutted process in circular depression (small arrow) from 343-b (138.3 m depth). **(d)** External fine structures on valve mantle and marginal area with arrows pointing to external openings like 'vestibules' of mantle strutted processes (same specimen as (a)).

the ring formed by the mantle strutted processes and has a short truncated conical tube externally (Fig. C2d) with a simple sessile slit parallel or slightly oblique to the valve margin internally (Fig. C2d). Spines not observed. Cribral structure unknown.

Stephanodiscus praesuzukii Saito-Kato sp. nov.
(Fig. C3a–d, f, g)

Holotype. MPC-03023 (Micropaleontology Collection, National Museum of Nature and Science, Tokyo).

Type locality. No. 223-c subsampled from 93.63 m depth of 200 m core, Lake Biwa, Shiga Prefecture, Japan.

Description. Valve discoid (Fig. C3a, b) ranging from 4 to 24 μm in diameter. Central area concave (Fig. C3a, c, d) or convex (Fig. C3b, f, g). Radiating areolar rows on the valve face form broad fascicles, with two rows at the valve centre and 3–4 at the valve margin (Fig. C3d, g). Interfascicles, i.e. costae, well developed and raised externally with complex connections to external openings of areolae (Fig. C3c, f). On the interfascicles of most valves

spines are arranged irregularly at valve face/mantle junction (Fig. C3c, f). Mantle strutted processes located on almost every interfascicle, forming a ring. The external openings of mantle strutted processes are short tubes (Fig. C3c, e).

Remarks. *Stephanodiscus praesuzukii* is a distinct fossil taxon, although it has the same combination of valve morphology as *S. pseudosuzukii* described by Tuji & Kociolek (2000). Later, Kato *et al.* (2003) considered *S. pseudosuzukii* as a synonym of *S. suzukii* because the valves of *S. pseudosuzukii* occur in the life stages of *S. suzukii*. Correctly, the valve morphology of *S. praesuzukii* is quite similar to that of small cells of *S. suzukii*; however, maximum diameter does not exceed 25 μm.

Stephanodiscus suzukii (Tuji & Kociolek, 2000)
Kato *et al.* emend.
(Fig. C3e, h–j)

Description. All *S. suzukii* specimens from the 200 m core exhibited discoid valves with raised costae (interfascicles) and
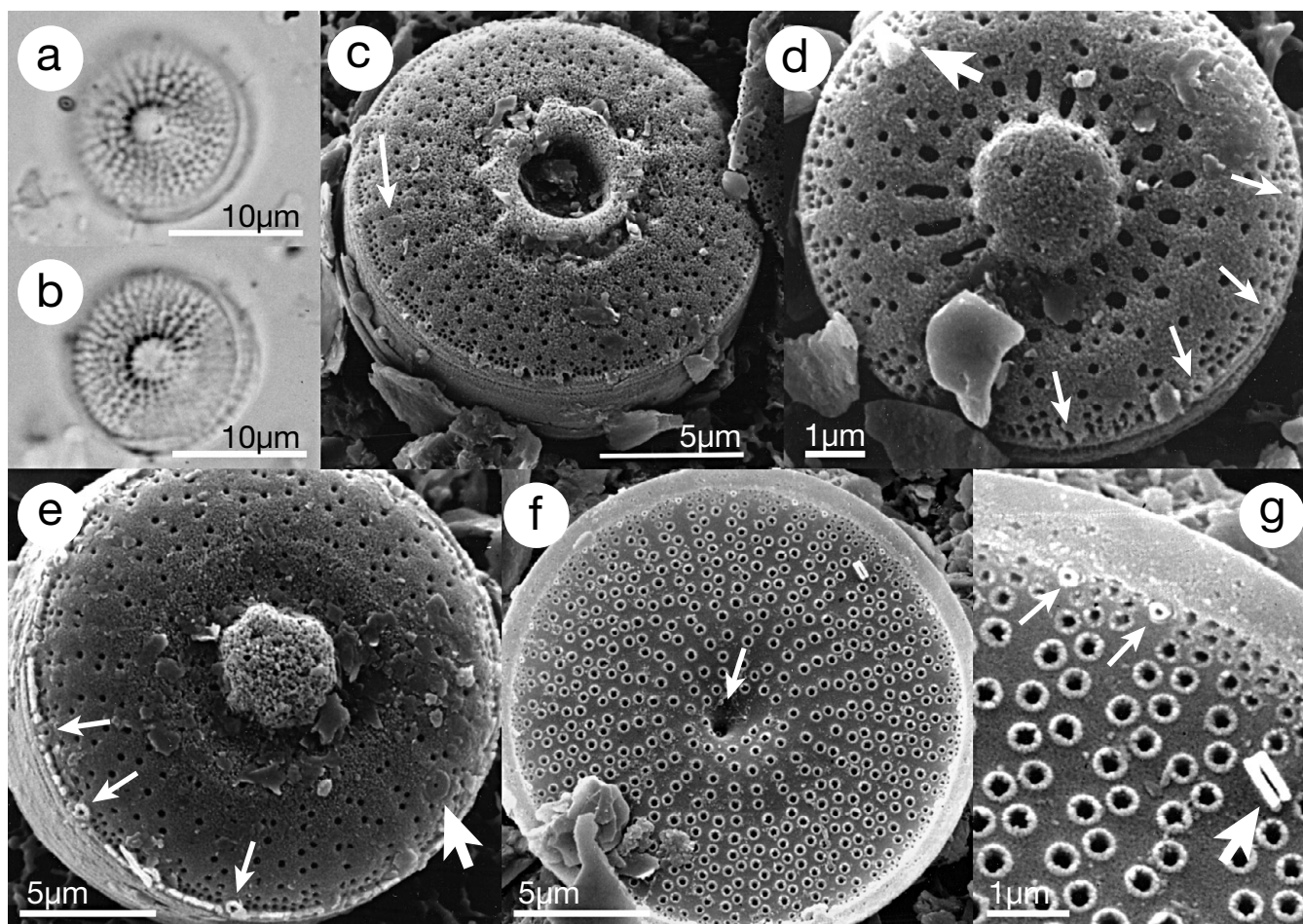
Fig. C2. LM and SEM images of *Stephanodiscus umbilicatus* sp. nov. from Lake Biwa 200 m core. **(a, b)** Pair valves of holotype from 315-b (127.7 m depth). **(c)** External view of concave valve with central depression and cast of labiate process tube (arrow) from 315-b (127.7 m depth).). **(d)** External view of convex valve with conspicuously raised central area, short tube of labiate process (large arrow) and small pores of external openings of mantle strutted processes (small arrows) from 319-d (129.4 m depth).). **(e)** External view of convex valve with conspicuously raised central area, cast of external tube of labiate process (large arrow) and rimmed pores of mantle strutted processes (small arrows) from 315-b (127.7 m depth).). **(f)** Internal view of convex valve showing sessile labiate process and central strutted process in depression (small arrow) from 315-a (127.5 m depth).). **(g)** Close-up image of (f) showing labiate process (large arrow) and mantle strutted processes (small arrows).).

depressed areolated areas (fascicles) (Fig. C3e, h), a radiating are-olar row system with one puncta in the centre increasing to four at the valve margin (Fig. C3i, j), generally a single central strutted process (Fig. C3j), a mantle strutted process on each interfasci-cle possessing three satellite pores, a labiate process on the valve mantle sessile in habit (Fig. C3j) or raised on a stalk (Fig. C3i depending on valve diameter, and short tubes for external open-ing of the mantle strutted processes and a longer and thicker tube for the labiate process (Fig. C3e, h). Spines were observed on 0–40% of the population at each horizon. Some specimens had well-developed bifurcate or forked spines (Fig. C3h).

Remarks. The maximum diameter reaches 50 μm which is con-sistent with values reported for modern *S. suzukii* populations (Tuji & Kociolek, 2000) and 1000-year-old fossil populations from Lake Suigetsu (Kato *et al*., 2003). Fossil specimens iden-tified as *S. suzukii* exhibited rather thick valve morphology; however, this was considered part of the species' intraspecific variation. Each geographical and temporal population of *S. suzukii* showed varying degrees of valve silicification; weakly silicified

valves occurred in fossil material from Lake Suigetsu (Kato *et al*., 2003) relative to those in this study and modern ones (Tuji & Kociolek, 2000) from Lake Biwa.

Stephanodiscus cf. *niagarae* Ehrenberg, 1845

Remarks. This taxon was commonly observed from 197.0 (bot-tom of the core)–170.3 m (361–263 ka) in the 200 m core. Dis-coidal valves with high mantle range between 20 and 100 μm in diameter (40–75 μm commonly). Valve face/mantle junction raised and centrally gently raised or depressed. Radiation areola rows form narrow fascicles, one row at the central part and two rows at the margin on valve face. Smaller areolae arranged in radiat-ing rows on high valve mantle. Interfascicles are slightly raised externally at depressed area outside of central area and inside of valve face/mantle junction. Mantle strutted processes located on end of almost every interfascicle in the middle of mantle. The external openings of mantle strutted processes simple pores under spines irregularly arranged in a ring on valve face/mantle junc-tion; the internal tubes of these short with three satellite pores
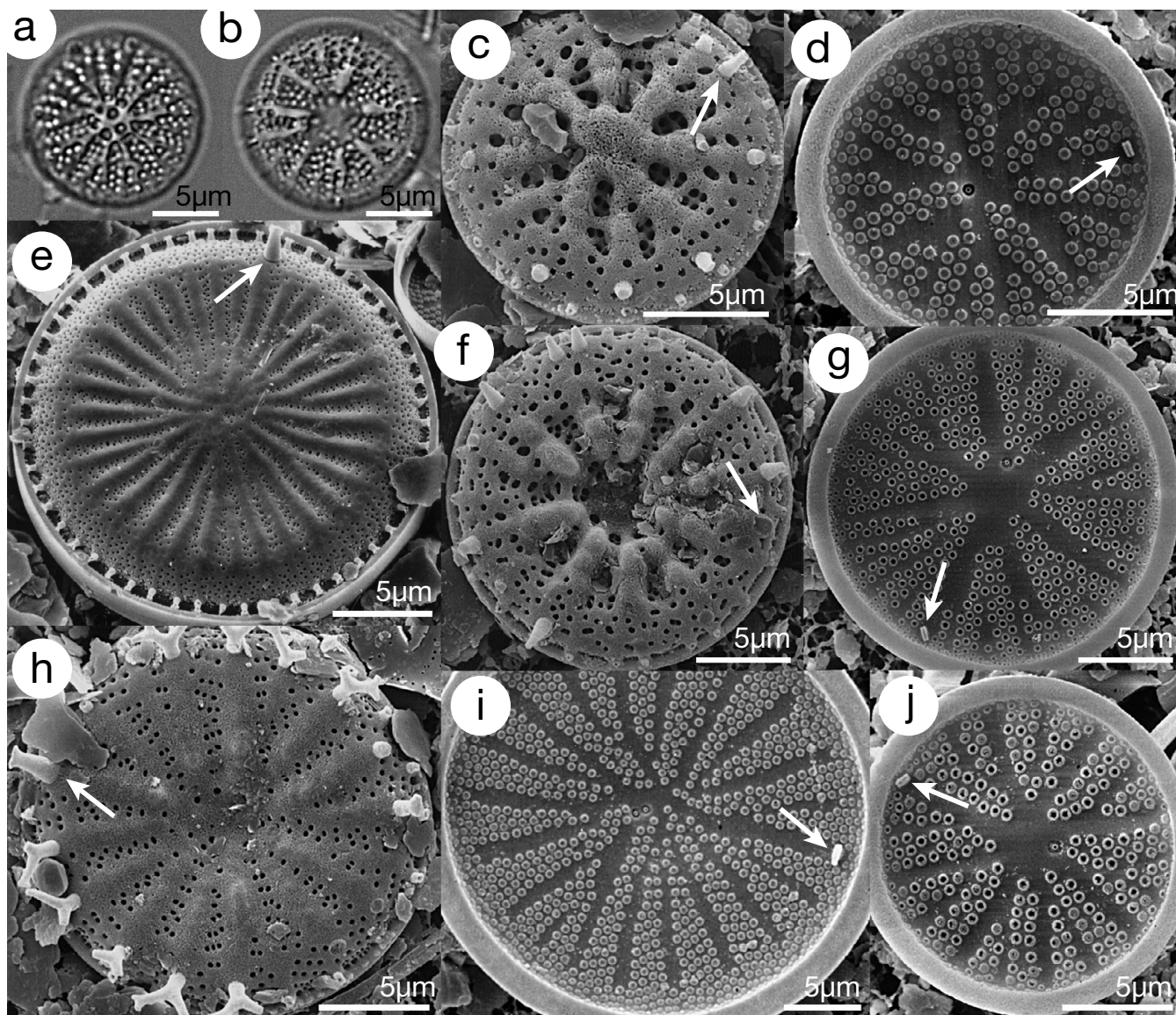
Fig. C3. LM and SEM images of *Stephanodiscus praesuzukii* sp. nov. and *S. suzukii* from Lake Biwa 200 m core. **(a-d, f-g)** *Stephanodiscus praesuzukii* sp. nov. **(a)** Concave valve of holotype and **(b)** convex valve from 223-c (93.6 m depth). **(c)** External view of convex valve with arrow pointing to external tube of labiate process from 217-a (92.0 m depth). **(d)** Internal view of convex valves with arrow pointing to sessile labiate process from 207-a (88.2 m depth). **(f)** External and **(g)** internal view of concave valves with arrows pointing to labiate process from 233-a (97.5 m depth). **(e, h-j)** *Stephanodiscus suzukii.* **(e)** External view of convex valve with no spine with external tube of labiate process (arrow) from 165-a (67.2 m depth). **(h)** External view of concave valve with Y-shaped marginal spines, arrow pointing to external tube of labiate process from 183-a (76.1 m depth). **(i)** Internal view of convex valve with stalked labiate process (arrow) and two central strutted processes in circular depressions from 183-a (76.1 m depth). **(j)** Internal view of concave valve and arrow pointing to sessile labiate process from 183-a (76.1 m depth).

covered by high cowlings. Valve face strutted processes distributed on a ring in large valves and more or less scattered in small valves. Several labiate processes located slightly outside of the strutted process ring with stalked slit oblique to valve margin internally; external structure of this may be small pore but we did not observe it. Domed cribra on valve face and mantle.

Our specimens from Lake Biwa 200 m core were generally very similar to *S. niagarae* Ehrenberg, a living species in North America, and had almost the same cell dimensions, fascicle, spine, mantle strutted process and labiate process density. Location of central

strutted processes in a ring, and three satellite pores surrounding most of them, are also a common character. Fossil material from the 200 m core did not have external tubes for mantle strutted processes, as in *S. komoroensis* described from Japanese Pleistocene by Tanaka (2000), but this may be a taphonomic effect. An obvious difference between the specimens from the 200 m core and *S. niagarae* is the position of labiate processes, that is slightly outer from the mantle strutted process ring in 200 m core specimens and slightly inner than this in *S. niagarae*. Further studies are required to compare our material with the modern North

American specimens, as well as Japanese fossil taxa such as *S. niagarae* reported from palaeo-Lake Biwa strata (Tanaka & Matsuoka, 1985).

Stephanodiscus cf. *oregonicus* (Ehrenberg, 1854; Håkansson, 1986)

Remarks. This taxon was observed in the section 197.0 (bottom of the core)–158.9 m (361–241 ka) in the 200 m core. Discoidal valves *c*. 5–20 µm in diameter dominant. Central area conspicuously convex or concave. Radiating areola rows form rather wide fascicles, one row at the central part and 3–4 rows at the margin on valve face. Smaller areolae on valve mantle. Interfascicles thick and raised externally. Some valves had hyaline ring(s) at valve face/mantle junction and/or just outside of central elevation or depression. Marginal strutted processes located on every, or every second, interfascicle at very end of valve margin. The external openings of marginal strutted processes are simple pores difficult to distinguish from mantle areolae; the internal tubes of these are short with three satellite pores covered with high cowlings. No central strutted process observed. A labiate process located on marginal strutted process ring with simple sessile slit parallel or slightly oblique to valve margin internally; the external structure could not be distinguished. A ring of irregularly arranged spines observed on interfascicles at valve face/mantle junction. Broken domed cribra observed.

The morphological characters of this taxon agree with the description of *Stephanodiscus* sp. A in Kuwae *et al*. (2007). Weakly silicified forms were observed from 197.0 (bottom of the core)–166.5 m depth (361–255 ka). From co-occurrence and morphological similarity but with larger diameter, the weakly silicified forms might represent younger vegetative stages in the life cycle. They also correspond to *Stephanodiscus* sp. B in Kuwae *et al*. (2007). Further research on the morphological distinctiveness of these forms will be reported elsewhere.

Aulacoseira nipponica (Skvortzov, 1936; Tuji, 2002) in relation to *A. nipponica* type-1 and type-2

Remarks. *Aulacoseira nipponica* occurred as a predominant species during interglacial periods. We identified its occurrence after MIS5 (above 75.0 m corresponding to 108 ka) as an extant species, *Aulacoseira nipponica* revised by Tuji (2002). Several valves were joined with long straight linking spines, each of which had a single areola row and each between them a single row on valve mantle with many granules. No areola was observed on valve face. Some specimens developed with ringleist, and others did not. Several labiate processes were located on the ringleist.

Slight morphological differences from *A. nipponica* were recognized in *Aulacoseira* type-1 from MIS 7 (140.1–113.2 m, 211–169 ka) and *Aulacoseira* type-2 from MIS9 (197.0–183.8 m, 361–313 ka). They were distinct in their valve size and diameter/height ratio; however, the other valve features, such as areola array, spine shape and location of labiate processes on ringleist, are the same. Many initial valves of *A. nipponica* occurred from 138.8 to 133.7 m in depth (207–201 ka), and from 70.8 to 38.3 m (950–513 ka). From these sections, weakly silicified valves were commonly observed. The oldest form of our material, *Aulacoseira* type-2, was characterized by diverse diameter/height ratio, wide and short and narrow and tall.

Based on similarities of valve morphology, these three *Aulacoseira* species should be closely related. Their stratigraphic distributions imply their phylogenetic relationships, just like the *Stephanodiscus* species in this article, although we only assume it because their intermittent occurrence did not allow us to examine intermediate forms among them. Further research is required to compare their valve morphologies with the very similar North American fossil, *Aulacoseira solida* (Eulenstein in Van Heurck, 1882; Krammer, 1991), which was discussed together with *A. nipponica* in Tuji (2002).

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