

First report of *Protokeelia spinifera* and *Rhopalodia iriomotensis* from China, with comments on their systematic positions

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Abstract *Protokeelia* is described as a new and rare pennate diatom genus in China. Its valve includes two parts: ventral valve and dorsal valve. The ventral valve is more or less convex or concave. The raphe runs in a distinct elevated and thickened siliceous ridge. In the internal valve, beneath the raphe are fibulae or fibulae clusters which connect the ventral and dorsal valves. One species, *P. spinifera* Round & Basson, is observed in the water samples from a mangrove forest in Sai Kung, Hong Kong, China. Its main morphological features are described in this paper along with electron microscope (EM) photos. Morphological comparisons are also made among several allied species. In addition, a newly recorded species for China, *Rhopalodia iriomotensis* Kobayasi, Nagumo & Tanaka, is discussed here. Because several key features observed in this species resemble those of some *Protokeelia* species, we suggest that it may actually represent a *Protokeelia* species.

Key words China, new genus record, *Protokeelia*, *Rhopalodia iriomotensis*.

Nanoplanktonic diatoms (Nanodiatoms) are important primary producers in marine waters and make a great contribution to diatom biomass and species diversity, especially in coastal waters (Fay, 1973; Hallegraeff, 1981; Gao, 1990; Cheng et al., 1993; Jiao & Gao, 1995; Li, 2006). Some nanodiatom species are key food source for many aquaculture organisms such as abalone, bivalves and junior fishes. Many nanodiatom species belonging to the genera such as *Skeletonema*, *Thalassiosira*, *Chaetoceros* and so on are the common and main bloom causative organisms in Chinese coast. They have been either frequently underestimated or overlooked in phytoplankton investigations due to their small size (2–20 µm), which allows most of them to pass through a regular phytoplankton net of 70 µm pore size. In the past ten years, the significance of nanodiatoms has been validated in several qualitative and quantitative studies using water samples instead of net samples in many coastal waters of China such as Xiamen Harbour (Gao, 1990), Fujian Province coastal waters (Cheng et al., 1993), Jiaozhou Bay (Jiao & Gao, 1995), Hong Kong waters (Gao et al., 2003; Li, 2006), Changjiang River Estuary waters (Li, 2006), Daya Bay (Li, 2006), etc. Many new species and new records of genera and species of nanodiatoms have been reported.

In the present study, we describe a new genus

record of nanodiatoms, *Protokeelia*, from water samples collected from several representative sea areas of Chinese coast from May 2002 to May 2006.

Protokeelia is a rare nanodiatom genus including six taxa which have been reported mainly in warm waters; the literature about this genus and its species is scarce. Fortunately, in this paper, one *Protokeelia* species, *P. spinifera* and an allied species, *Rhopalodia iriomotensis*, were found in the water samples from a mangrove forest in Sai Kung, Hong Kong, China.

1 Material and methods

Two liters of water samples were collected from a mangrove forest in Sai Kung, Hong Kong, China on 18 December, 2004 during the period of high tide. The sites of collection were approximately mid-intertidal.

All water samples were fixed with Lugo's solution *in situ* and brought back to the laboratory. The preserved samples were concentrated to a final volume about 5 mL by settlement overnight repeatedly. Then they were acidized with concentrated H₂SO₄ by boiling for 20–30 min in a water bath and rinsed with distilled water to neutrality. Afterwards they were observed and photographed on the carbon-coated copper under HITACHI H600 TEM, or coated with gold before observation under PHILIPS L30 SEM (Cheng et al., 1993; Gao et al., 2003).

The samples were deposited in Diatom Laboratory of Xiamen University (DLXU), numbered as 041218HK series.

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2 Results

Protokeelia spinifera Round & Basson in Diatom Research 9 (1): 156, figs. 2–25. 1994.

具刺原龙骨藻 Figs. 1–8

Cells appear like orange-segment and the valve is lunate, with the raphe system running in an elevation from apices to the center separating a wide ventral valve face from a narrow dorsal valve face. The valve is 5.8–10 μm long and 3.6–5.5 μm wide. Except the dimensions, they have several key characteristics that are the same as in many larger specimens observed by Round and Basson in 1994 (10–18 μm long and 7–9 μm wide). The typical characteristics of this species are triple-ridges on the ventral valve face (white arrows in Fig. 1). On the exterior valve, the ridge lying adjacent to the raphe elevation is reduced or flattened at the center (white triangle in Fig. 3), corresponding to a plain section (white arrows in Fig. 4) on the interior valve. The mid-ventral valve face ridge extends across the valve face, with the third ridge lying close to the ventral valve margin. The dorsal valve face is concave (almost U-shaped) and distinctly ribbed (interstriae) (white arrow in Fig. 7). The raphe sits running a raphe sternum forming a smooth arc from valve apex to apex, with a sinkage near the center (white triangle in Fig. 3). Apical endings are simple (white arrows in Fig. 2). Central endings are not shown in our photos, and they are described as widely spaced, slightly curved to the dorsal side in the previous papers. With the densities about 18–22 in 10 μm , striae are composed of two rows of areolae, occasionally one more row of small areolae at the middle area between them. The shapes of areolae are mainly poroids, but some of them are long oval or rectangle in our specimens (shown in Fig. 8). In the internal valve view, there is a small patch of areolae (black triangle in Fig. 4) adjacent to the centre of the raphe sternum. Interstriae are slightly raised, often bifurcating towards the raphe sternum (which are clear in Fig. 8). Small spinules (white arrows in Fig. 3) occur on the interstriae particularly along the ventral valve face ridges and are scattered on the face adjacent to the concavity. Spinules may be robust and obvious in some valves, but sometimes they are not so distinct. Internal fibulae (black arrows in Figs. 4, 5) extend beneath the raphe sternum elevation except near the apices and along a central section. Adjacent to the apices the valve face dips down producing a hollow region (white triangles in Figs. 4–6) at the internal apices.

Habit: It has been reported by Round and Basson in 1994 that *P. spinifera* lives in the sediments such as coarse sand and muddy sediments. Our water samples were collected from a mangrove forest. It should be benthic and tychoplankton because of water mix.

Distribution: This species was first discovered in the sediments from Saudi Arabia by Round and Basson in 1994. Our materials were several water samples collected from the mangrove forest in Sai Kung, Hong Kong, China in 18, December 2004.

In the same sample as *P. spinifera*, a species resembling to *P. spinifera* but identified as *Rhopalodia iriomotensis* by Kobayasi et al. in 1993 was also observed.

Rhopalodia iriomotensis Kobayasi, Nagumo & Tanaka in Nova Hedwigia, Beiheft 106: 134, figs. 1–30. 1993.

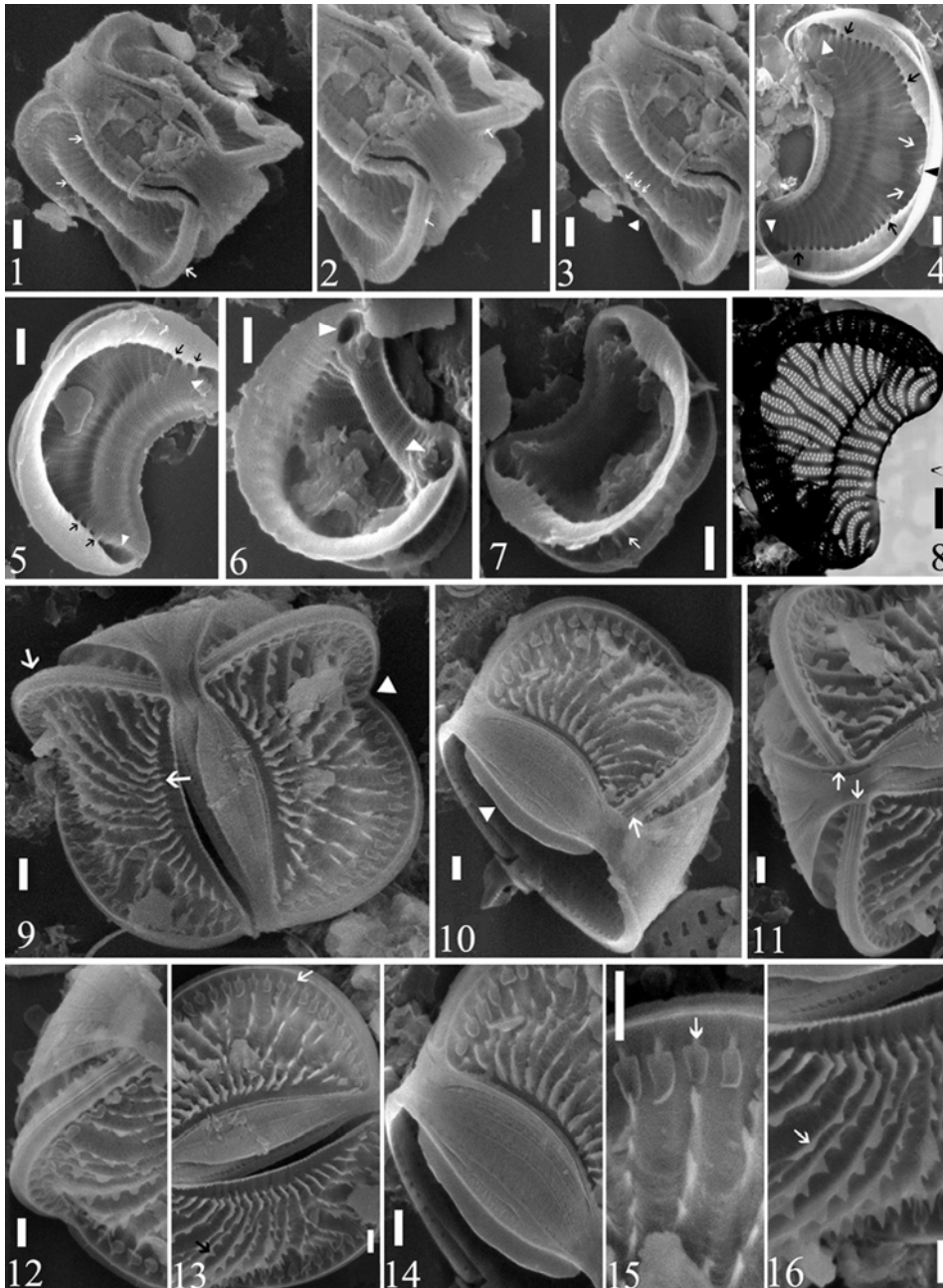
伊岛棒杆藻 Figs. 9–16

Cells are almost quadrangular or orange-segment-like (Figs. 9, 10). Valves are strongly dorsi-ventral. The dorsal margin is strongly convex (upper white arrow in Fig. 9), and the ventral margin is slightly concave or straight and clearly inflated at the center. Apices are obtusely rounded and usually ventrally bent, 10–25 μm long, 5.5–10.5 μm broad. The ventral valve may be smoothly convex or flat near the center. Near the ventral margin runs an arched apical costa (middle white arrow in Fig. 9). The raphe runs along in a raised sternum and sinks at the center (white triangle in Fig. 9). Primary fibular costae are distinct but curved and sometimes are branched, about 4 in 10 μm . The densities of striae are 11–12 in 10 μm .

The cingulum is strongly dorsi-ventral with a narrow ventral side and a broad dorsal side. It is composed of several indecipherably open bands (seven bands observed by Kobayasi et al., 1993), the number of which could not be discerned clearly in our specimens. The ventral septum (white triangle in Fig. 10) is distinct and has a broad expansion towards the inside which spreads out onto the arched apical costa of the ventral valve.

Habit: Attached on the intertidal roots of mangroves.

Distribution: It was first reported in the samples scraped from the surface of mangrove roots growing on the riverside in Iriomote Island situated in the southernmost part of Japan. Our materials were several water samples collected from the mangrove forest in Sai Kung, Hong Kong, China on 18 December, 2004.



Figs. 1–8. SEM (1–7) and TEM (8) photographs of *Protokeelia spinifera*. **1.** Orange-segment cell shape. Stress on the ventral marginal (upper arrow), central (middle arrow) and dorsal marginal (lower arrow) ridges. **2.** Enlargement of the cell end, showing simple raphe apices (white arrows) along in the sternum. The copulae have collapsed between the valves. **3.** Focus on the external ventral valve face, with few indecipherably small spinules (white arrows) occurring on the central ridge. Sinkage (white triangle) occurs near the center of raphe sternum and dorsal ridge. **4–7.** Internal valve views. **4.** Keystone on the fibulae regions (black arrows), one side of which is adjacent to a plain section (white arrows) near the center of the raphe sternum and the other side is joint with a hollow region (white triangles) at the internal apices. A small patch of areolae (black triangle) is near the center of raphe sternum. **5, 6.** Similar to Fig. 4. Focus on the fibulae (black arrows) and hollow regions (white triangles). **7.** Show the part of the dorsal valve with interstriae (white arrow). **8.** Indicate the shapes of areolae, mainly poroids and partly long oval or rectangle. The black line occurring in the middle of the valve is the central ridge on the external valve face. Interstriae often bifurcate towards the raphe sternum. **Figs. 9–16.** SEM photographs of *Rhopalodia iriomotensis*. **9.** Orange-segment-like cell shape. Only two ridges on valve face could be observed clearly, including strongly raised dorsal marginal ridge (upper white arrow) with an obvious sinkage at the center (white triangle), arched apical costa close to the ventral valve margin (middle white arrow). **10.** A cell without a valve. Focus on the raphe end (white arrow) and ventral septum (white triangle). **11, 12.** Show the simple apical endings of raphe (white arrows). **13.** View of two ventral valves, stressing on two key characteristics, shoehorn-shaped projections (white arrow) and the raised costae blade-like ridges (black arrow). **14.** Show the copulae and the arched apical costa. **15.** Enlargement of the shoehorn-shaped projections (white arrow). **16.** Enlargement of the raised costae blade-like ridges (white arrow). Scale bars=1 μm .

3 Discussion

From the three key characteristics observed, it is confirmed that this taxon we found is the species *R. iriomotensis* described by Kobayasi et al. in 1993. The first is the shoehorn-shaped projections (white arrows in Figs. 13, 15) hanging from the canal raphe sternum. The positions of the projections correspond to the primary or secondary fibular costae. The shapes of the shoehorn-shaped projections are club-like at the ends of the canal raphe but they are terminally expanded and spatula-like at the other locations. The second feature is the raised costae blade-like ridges (black arrow in Fig. 13 and white arrow in Fig. 16) on the primary fibular costae of the ventral valve faces. The third feature is the robust and arched apical costa (middle white arrow in Fig. 9) which runs close to the ventral valve margin.

Only two specimens of “*R. iriomotensis*” were obtained, and no internal valve view was photographed (which has been shown clearly by Kobayasi et al., 1993). Because the three key features (described in the last paragraph) on the external valve of our specimens are identical to the main characteristics of *R. iriomotensis* described by Kobayasi et al., we conclude that they should be the same species. Therefore, the ample features on internal valves described by Kobayasi et al. could be cited and discussed here. In the description by Kobayasi et al. (1993), two kinds of features considered as the representative characteristics of the genus *Protokeelia* were given, fibulae beneath the raphe sternum and hollow region locating at each apex. We then suggest that *R. iriomotensis* might be a species of *Protokeelia*. Besides, it has a suite of characters that suggest a relationship to the genus *Protokeelia*. With the same shape as *Protokeelia* species, *R. iriomotensis* has a rather lunate or orange-segment-like cell shape, which is not so typical in *Rhopalodia* species. Another key characteristic is the strongly raised raphe sternum at the dorsal margin of *R. iriomotensis*. It even protrudes much higher than several *Protokeelia* species, a feature not so obvious in any *Rhopalodia* species. Furthermore, in view of other features, such as slightly convex at the middle ventral valve and arched apical costa close to the ventral valve margin, we suggest that “*R. iriomotensis*” may be a species of *Protokeelia*, *P. iriomotensis*.

The external valve features of “*R. iriomotensis*” are very similar to those of *P. aculeata* Round & Basson. Their common features are orange-segment-like cell shape, more elevated raphe sternum, slightly convex at the middle ventral valve, conspicuous bifurcate spines distributing on the ventral valve and

internal ribs running across the internal ventral valve, crossing the raphe canal and continuing onto the dorsal valve face (it was not mentioned in the paper of Kobayasi et al. in 1993 but clear in their photos). One key difference is the shoehorn-shaped projections hanging from the edges of conopea on both sides of the canal raphe in “*R. iriomotensis*”, which instead are like fingers or long spines extending from either side of the sternum in *P. aculeata*.

Protokeelia spinifera differs from other species of this genus by the following features: the rows of spinules on the ventral part of the valve face, the more concave ventral and convex dorsal margins of the valve, the smoothly convex sternum bearing the raphe, having no clustered fibulae below the raphe, the ridging of the ventral valve face including a central break on the ridge adjacent to the centre of the raphe sternum, and the arc of curvature of the valves (Round & Basson, 1994).

Protokeelia was first described as a genus by Reimer and Lee in 1984, based on *P. hottingeri*, found in the Gulf of Eilat and Queensland, Australia. A second species, *P. bassonii*, was observed by Round (1993) in the material collected on tidal flats on the west coast of Bahrain, Persian Gulf. The original taxon, *P. hottingeri*, was found as a symbiont living in association with Foraminifera. The second taxon, *P. bassonii*, was free-living. From then till now, other four new *Protokeelia* species have been identified by Round and Basson from 1993 to 1995. Among them, two were newly observed species (Round & Basson, 1994, 1995b) and two were revised and transferred from *Auricula* to *Protokeelia* after reexamination on the original samples (Round & Basson, 1995a). In all these six *Protokeelia* species, two transferred species were observed in the samples of Giffen which have been collected from the Ngwenyana River in Eastern Cape Province, South Africa. The other four species have been found from only two sites, Red Sea (Israel, Bahrain, Saudi Arabia) and Australian waters (Great Barrier Reef). The holotype, *P. hottingeri*, is cultured Foraminiferal (*Amphistegina lessonii*) endosymbionts. It has been demonstrated that many of these Foraminiferal endosymbionts are actually protoplasts of diatoms. And upon culturing, cells with silicon walls were formed. Other five reported *Protokeelia* species are free-living in warm waters. Several species have been found from mangrove forest waters, which indicated that they are mainly living attached on the roots of mangroves or in the muddy sediments.

Protokeelia Reimer & Lee in Proceedings of the Academy of Natural Sciences, Philadelphia 136: 195.

1984; Round in *Diatom Research* 8 (1): 203. 1993; Round & Basson in *Diatom Research* 9 (1): 156, figs. 2–25. 1994, in *Diatom Research* 10 (1): 212, figs. 1–9. 1995, in *Diatom Research* 10 (2): 333, figs. 1–15. 1995.

原龙骨藻属

The valve is semi-lunate or orange-segment in shape, with ends varying from distinct and narrowly rostrate to broadly rounded and hardly differentiated. It is composed of two parts, the main and larger valve is called ventral valve, semi-lunate, and the smaller valve is called dorsal valve, with narrow strip and nearly U-shaped. The raphe branches near the dorsal margin of the raphe-side, especially straight, on a distinctly elevated and thickened siliceous ridge. Marginal siliceous thickenings and valve surface depressions are visible both on the residuum and the raphe-sides of the frustule. Striae are more or less parallel and doubly punctate. Puncta are discernable only under SEM as simple poroids.

The shape of *Protokeelia* species is so complex and delicate. Many similar morphological features are shared among several genera. When viewed under the light microscope, Reimer and Lee (1984) had to reconsider its assignment to the genus *Amphora*. However, after SEM observation, they thought that no species in the genus *Amphora* shows the raphe branches relatively straight and along the dorsal margin together with surface depressions, beneath which are the fibular clusters supporting a barely occluded cavity (caverna). So they believed that this taxon must be excluded from *Amphora* as presently defined.

With the crescent-shaped valve or orange-segment-like cell, *Protokeelia* is so similar to *Auricula* and *Rhopalodia* (Round et al., 1990; Round & Basson, 1994). The other common features are raphe running along the dorsal margin, main striae biseriate, fibulae short, rib-like, arising from the two ridges flanking the raphe internally. There are several distinct features between *Protokeelia* and other two genera: *Protokeelia* has an obvious ridge lying adjacent to the raphe elevation and the valve face is more concave or convex. In comparison, the raphe sternum and valve face of *Auricula* and *Rhopalodia* are flat, though the raphe sternum of *Rhopalodia* is more protuberant than in *Auricula*. Several taxa can be confused with each other and two *Auricula* species have been transferred to *Protokeelia* after reexamination of the original materials. *Protokeelia* has rather similar features with *Rhopalodia*, but it has in general a more lunate form and a more pronounced/raised raphe sternum than

Rhopalodia. Also, as pointed out by Reimer and Lee (1984), the internal costae and distinct oval internal openings below the raphe system do not fit into the *Protokeelia* mould.

Protokeelia was placed in the family Auriculaceae by Reimer and Lee in 1984 due to their general features, such as the arrangement of the areolae. However, it differs from *Auricula* in the shape of valve, the moulding of the valve on either side of the raphe system, and in the formation of fibular clusters. After more and detailed observations, Round and Basson suggested that it might be assigned to the family Rhopalodiaceae because of the lunate form and the protuberant raphe sternum. And it is recommended that much more details are urgently needed before a more complete discussion of the systematics can be conducted (Round, 1993).

When the first species, *P. hottingeri*, was found and the genus *Protokeelia* was established, Reimer and Lee defined the principle features on the genus level as: fibular clusters and the widely spaced surface depression near the dorsal margin, which correspond to the internal attachment points of the fibular clusters. Later, several new and transferred species were reported by Round and Basson. The diagnostic features of the genus have been changed: fibular clusters instead of fibular, and surface depression instead of convex or concave distinctly or slightly. *Protokeelia* has in general a more lunate form and a more pronounced/raised raphe sternum than *Rhopalodia* has. Nevertheless, both of these features are subjective. Therefore, better defined differences between *Protokeelia* and *Rhopalodia* or other allied genera should be sought in future studies.

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