

A new record of rare dinoflagellate species *Protoperidinium carum* (Abé, 1981) Balech, 1994 for Vietnamese waters and Southeast Asia region

Phan Tan Luom^{1,2}

¹*Institute of Oceanography, VAST, Vietnam*

²*Graduate University of Science and Technology, VAST, Vietnam*

E-mail: luom.dt@gmail.com

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Abstract

Although there were a number of studies on the section *Oceanica* of the genus *Protoperidinium*, most of the published figures did not well revealed the clear features of the anterior intercalary plates. In this paper, *Protoperidinium carum* was re-described in details of cell morphology, plate patterns and thecal ornamentation, especially the second anterior intercalary plate (2a) is wide pentagonal instead of hexagonal type as in the previous descriptions. This species was illustrated with line drawing, light and scanning electron microscope photographs. *P. carum* was newly recorded in Southeast Asia and Vietnamese waters.

Keywords: *Oceanica*, *Protoperidinium carum*, new finding, Southeast Asia, Vietnam.

INTRODUCTION

Protoperidinium is the largest genus of heterotrophic thecate dinoflagellates and occurs in all the world's oceans [1], and it has a long, complex taxonomic history [2]. Most of the *Protoperidinium* species formerly belonged to genus *Peridinium* Ehrenberg 1830, which included marine and freshwater organisms. Bergh (1881) established the genus *Protoperidinium* which included species with distinct sulcal lists but leaving in *Peridinium*, those with antapical horns or without sulcal list [3].

Balech (1974) when re-instating the genus *Protoperidinium* Bergh (1881), had transferred 231 marine species of *Peridinium* Ehrenberg (1830) to the *Protoperidinium* which included marine species with 4 cingular plates or 3 cingular and 1 transitional (t) plates. The remaining freshwater species of genus *Peridinium* were with 5 or 6 cingular plates. Furthermore, he subdivided the genus *Protoperidinium* into three subgenera based on the number of apical intercalary plates and precingular plates: *Minusculum*, *Archaeperidinium*, and *Protoperidinium* [4]. He simultaneously further subdivided *Protoperidinium* based on the criteria used by Jörgensen (1912) and Paulsen (1931). Faust (2006) erected the new subgenus *Testeria* Faust for *Protoperidinium* species with seven precingular plates, apical plate 1' disconnected from pointed, one intercalary plate and no apical pore complex [5].

In Southeast Asia, there were 64 species of *Protoperidinium* with four of them belonging to the section *Oceanica* reported mainly in the Gulf of Thailand and East Coast of Peninsular Malaysia [6, 7], Sabah, Sarawak, and Brunei Darussalam [8, 9], Western Philippines [10], Vietnamese waters [11, 12], and Cambodian waters [13]. However, most of them are included in the lists of phytoplankton species without illustrations and descriptions.

In Vietnam, Hoang (1963) described and illustrated by line drawings 14 species of *Peridinium* (later *Protoperidinium*) from Nha Trang bay [14]. Shiota (1966) conducted a study of marine and freshwater phytoplankton

in Southern Vietnam, from Thua Thien-Hue to Rach Gia provinces, listing 14 species of *Peridinium* (later *Protoperidinium*) [15], and a total of 24 species of *Protoperidinium* were recorded from waters of Cat Ba National Park and Ha Long bay by Chu Van Thuoc et al., (1997) [16]. Later, the studies of the phytoplankton community were carried out in the South Central Vietnam, such as Nguyen Thi Mai Anh and Ho Van The (2001) [17], Nguyen Ngoc Lam et al., (2002, 2006) [18, 19], Ho Van The and Nguyen Ngoc Lam (2006, 2009) [20, 21]. From those, 14 species of *Protoperidinium* were recorded, with three of them belonging to section *Oceanica*.

Members of the section *Oceanica* are characterized by the first apical plate (1') of the ortho-type. The second anterior intercalary plate (2a) originally defining the section is quadra-type but can also be penta- or hexa-type [22, 23]. The epitheca ends in an apical horn of varying size and shape. The hypotheca has two well-developed hollow antapical horns with or without spines. The cingulum is descending, often markedly oblique relative to the longitudinal axis, and placed high on the dorsal side [24].

In recent years, a number of studies on taxonomy of the genus *Protoperidinium* in Vietnamese waters were carried out such as species diversity of the subgenus *Protoperidinium*, section *Oceanica*, ten species of which belonging to the section *Oceanica* were described with a new species *P. larsenii* and two new combinations *P. oceanicum* f. *bisintercalares* and *P. oceanicum* var. *tenellum* [24]; species diversity of sections *Conica*, *Tabulata* [25], and subgenus *Archaeperidinium* [26], and new record *P. anomaloplaxum* for Asia-Pacific [27].

Protoperidinium carum (Abé, 1981) Balech, 1994 was first described by Abé in 1981 (as *Peridinium carus*) based on rare specimens from Asamushi, Amori bay, Japan [28]. Description of the species was mainly on cell shape and some plate patterns with four drawings (fig. 46: 303–306). These figures did not well revealed the important feature of

Protoperidinium species, e.g. plate 2a. Balech (1994) transferred the species into genus *Protoperidinium* with neither additional descriptions nor illustrations [29]. The species has not been found anywhere else since then.

In this paper, *Protoperidinium carum* (Abé, 1981) Balech, 1994 is re-described and illustrated by line drawing, light, and scanning electron microscope (SEM) photographs to show details of its morphology, plate patterns, and thecal ornamentation.

MATERIALS AND METHODS

Sampling

Phytoplankton samples were collected with vertical net hauls (20 μm mesh size and net diameter of 30 cm) from near the ocean floor to the surface in different locations of Vietnamese coastal waters (from the Gulf of Tonkin to the Gulf of Thailand) (fig. 1). Samples were preserved with formaldehyde 5% in 25 ml dark glass bottles, and stored at the Institute of Oceanography, Nha Trang, Vietnam.

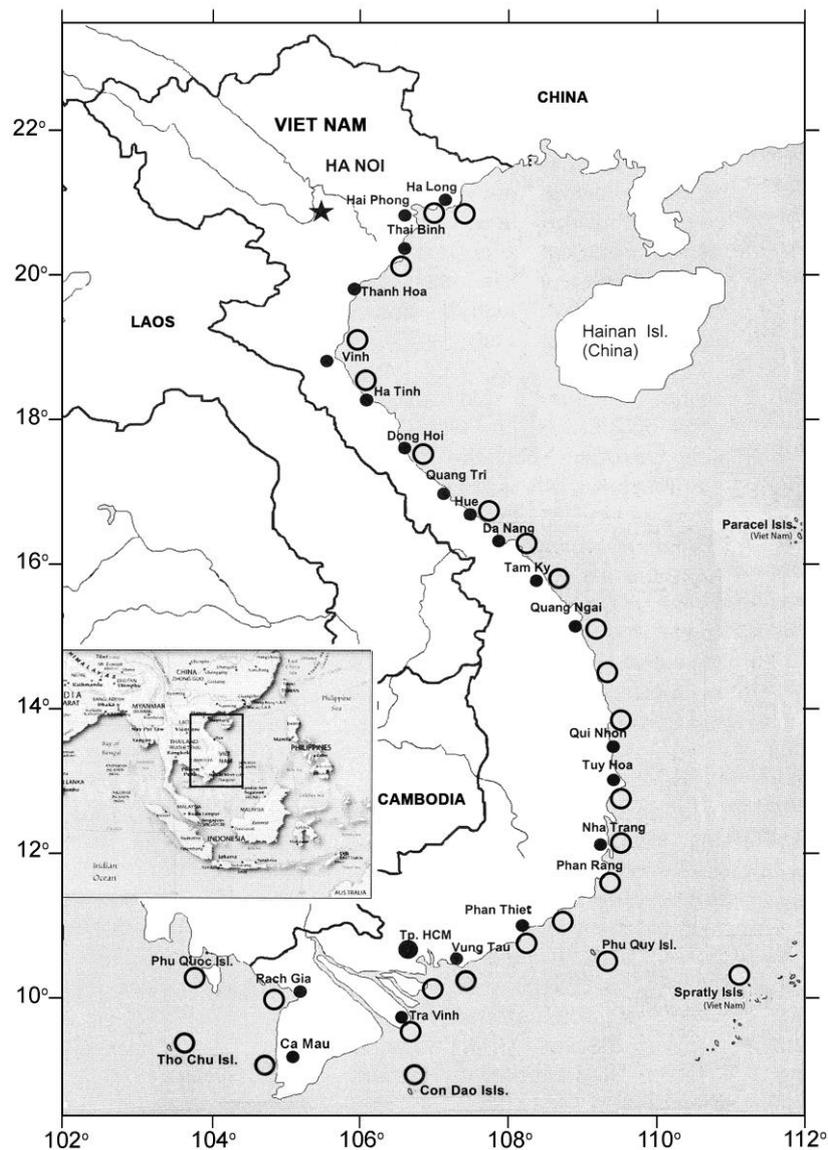


Figure 1. The map shows the position of sampling areas of Vietnamese waters: Provincial names (filled circles) and sampling areas (open circles)

Analyses of samples

The samples were examined by light microscope (LM) with phase-contrast (PC), differential interference contrast (DIC), and epifluorescence (Epi) optics. Observations of plate patterns were made with Calcofluor White M2R according to Fritz & Triemer (1985) [30]. A digital camera Olympus DP71 was used for light and epifluorescence microphotography.

For SEM examination, cells of *Proto-peridinium* were isolated by Pasteur pipette from preserved samples under a stereomicroscope and placed on a 5 µm carbon membrane filter, rinsed with distilled water, dehydrated through an ethanol series 15%, 30%, 50%, 70%, 2x 96%, and 2x absolute ethanol and air-dried. The filter was mounted onto an aluminum stub with carbon tape and finally sputter coated with gold. The stubs were examined on a Hitachi FE-SEM (Field Emission-Scanning Electron Microscope) model S4800 at Institute of Materials Science, VAST, Hanoi, Vietnam.

Thecal plate terminology follows the Kofoidian tabulation system [31]. The identification of the *Proto-peridinium* species and use of biometric terminology and abbreviations were based on Abé (1981) [28], Balech (1994) [29].

RESULTS

International nomenclature

Class Dinophyceae Pascher, 1914

Order Peridinales Haeckel, 1894

Family Proto-peridiniaceae Balech, 1988

Genus *Proto-peridinium* Bergh, 1881

Subgenus *Proto-peridinium* (Gran) Balech, 1974

Section *Oceanica* Jörgensen, 1912

***Proto-peridinium carum* (Abé) Balech, 1994; Figures 2a–2f, 3a–3f, 4a–4c**

Basionym: *Peridinium carus* Abé, 1981

References: Abé (1981, p. 326, fig. 46: 303–306) [28]; Balech (1994, p. 65, 78) [29].

Species description: Cell medium-size, about 117–120 µm long, 60–65 µm wide, and

28–30 µm depth, with the pentagonal body and the sides of the epitheca and hypotheca slightly concave (figs. 2a–2d, 3a–3b, 4a–4b), strongly dorso-ventrally compressed (figs. 2e–2f, 4c). Epitheca tapering into a robust short apical horn which was slightly bent to the left (figs. 2a–2d, 3a–3b, 4a–4b), and there was a low membrane along the sutures of apical plates (fig. 3f). Ortho-penta (1', 2a) plate arrangement. The first apical (1') plate was wide rhomboid and slightly asymmetrical with the anterior margins longest (figs. 2b, 3a, 4a). The second intercalary (2a) plate was wide pentagonal (figs. 2d, 3b, 3d, 4b). The cingulum was wide and oblique (figs. 2e–2f, 4c), descending about 1.0–1.25 times the cingular width (figs. 2a–2b, 3a, 3c, 4a), and bearing narrow smooth cingular lists (figs. 2b, 3a–3d, 4a–4b). Four cingular plates were present 4C (3+t) with very narrow transitional plate (t). The first cingular plate (C1) was narrower than C3 (figs. 3a, 3c), while the C2 plate is the widest and surrounding more or less the whole cell (fig. 2d). Hypotheca with two robust pointed antapical horns and great distance between the slightly divergent antapical horns (figs. 2a–2d, 3a–3b, 4a–4b). The sulcus was slightly oblique, deep and wide, becoming slightly wider posteriorly. A saw-edged membrane posteriorly surrounded the sulcal area (figs. 2a–2c, 3a–3c, 4a–4b). The anterior sulcal plate (Sa) extended onto the epitheca (figs. 2b, 3a, 3c, 4a), lined by sulcal lists that are connected to the cingular lists (figs. 2b, 3a, 3c). The shape of the post-cingular 1''' and 5''' plates were a brace-shaped (figs. 2b, 3c, 4a). The surface of the main thecal plates were ornamented by fine reticulations with spines at the junctions (figs. 3a–3e) and h pores of the varying size (figs. 3e–3f); whereas the surface of the sulcal plates was smooth with spores (figs. 3c, 3e).

Distribution: *Proto-peridinium carum* was first recorded in Asamushi waters (Japan) but extremely rare (Abé, 1981) [28]. However, it was commonly found in the coastal Vietnamese waters (such as Ha Long bay,

coastal Hai Phong, Lang Co lagoon, Nha Thuan province).
Trang bay, Cam Ranh bay, and coastal Ninh

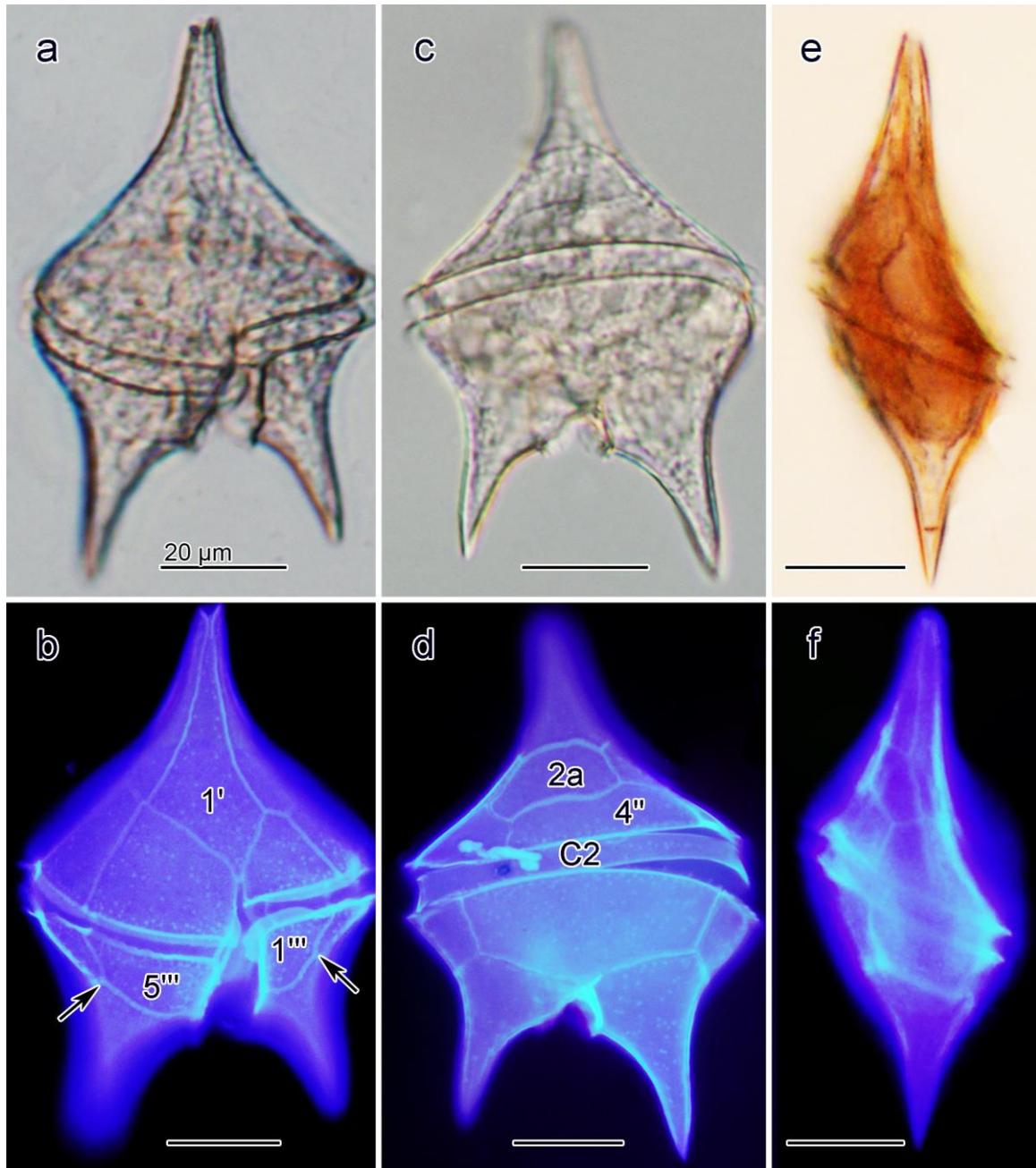


Figure 2a–2f. Light microscope of *Protoperidinium carum*: a (PC) and b (Epi) cell in ventral view showing the pentagonal cell body with the strongly apical and antapical horns, descending cingulum, the deep sulcus, the 1' plate, and showing the shape of the 1''' and 5''' plates with a brace-shape (arrows in fig. 2b); c (PC) and d (Epi): cell in dorsal view showing the wide pentagonal 2a plate, plate 4'', and C2; e (PC) and f (Epi): cell in right lateral view showing dorso-ventrally compressed and oblique cingulum. All scale bars = 20 µm

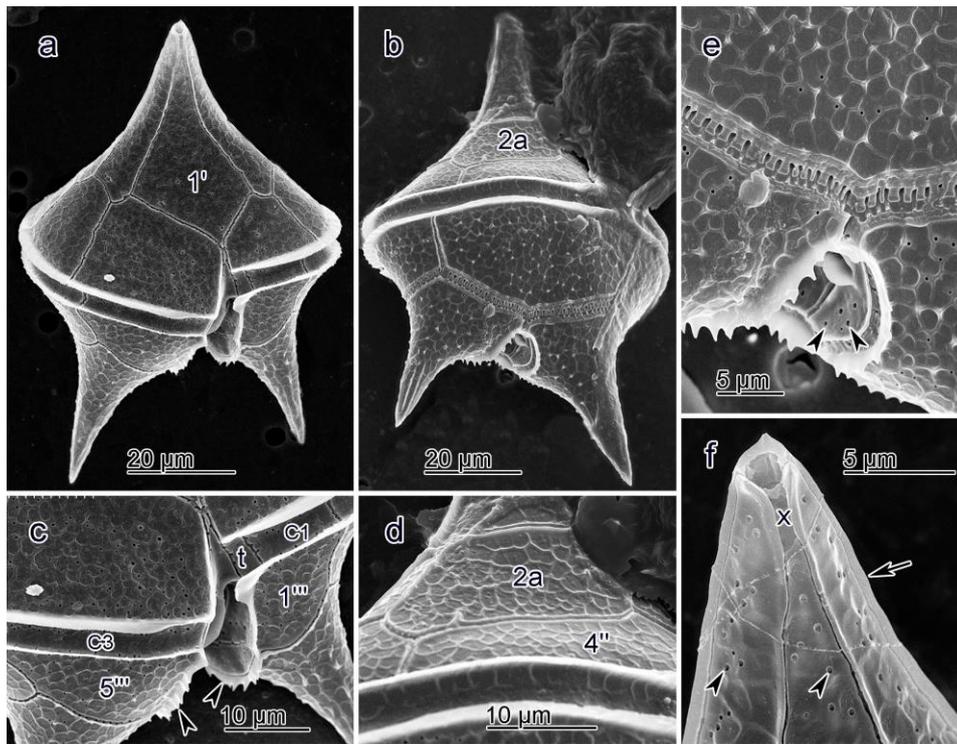


Figure 3a–3f. Scanning electron micrographs of *Protoperidinium carum*: a and c: cell in ventral view showing the cell body with the strongly apical and antapical horns, the wide 1' plate, descending cingulum (fig. 3a), the cingular plate patterns, the shape of the 1''' and 5''' plates, and the deep sulcus with two sulcal saw-edged lists (fig. 3c); b and d: cell in dorsal view showing the wide pentagonal 2a plate; e: part of hypotheca showing the reticulation with short spines at the junctions, and the smooth surface of sulcal plates with pores (arrowheads); f: a part of apical horn showing the canal plate (x), round pores (arrowheads), and low membrane along the sutures of apical plates (arrow)

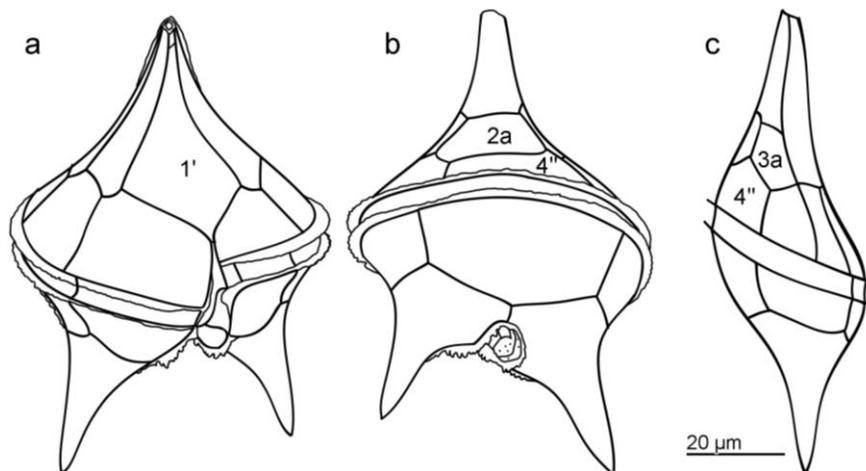


Figure 4a–4f. Line drawing of *Protoperidinium carum*: a: cell in ventral view; b: cell in dorsal view; c: cell in right lateral view showing dorso-ventrally compressed and oblique cingulum. Scale bar in fig. 4c = 20 µm applied to fig. 4a–4b

DISCUSSIONS

The cells identified as *P. carum* are in agreement with the original description and illustrations by Abé (1981, p. 326–327, fig. 46: 303–306) [28] with regard to cell shape, strong apical and antapical horns. According to Abé (1981), the middle intercalary plate (2a) was broadly hexagonal, but his illustrations only showed a part of the 2a plate in the left lateral view (fig. 46: 34) but not the dorsal view. This was not certain since the 2a plate would not be the same in the right lateral view. Observations on specimens ($n > 50$) from Vietnamese waters show almost all cells have wide pentagonal 2a plate. Additionally, the specimens from Vietnamese waters are larger (length 117–120 μm , width 60–65 μm , and depth 28–30 μm) than Abé's specimen (length 85–93 μm , width 45–48 μm , and depth 20 μm).

P. carum has distinct morphological features with other members of section *Oceanica*. However, it may be confused with *Protoperidinium venustum* (Matzenauer) Balech, 1974 (see in Phan-Tan et al., (2017), p. 142, figs. 10a–10e) [24], due to its cell outline and shape including: 1) a more angular cell shape, 2) strongly dorso-ventrally compressed, 3) strong antapical horns. However, it can be distinguished by the shape of the intercalary plates: quadra 2a plate in *P. venustum* and penta-type in *P. carum*; and *P. carum* has a greater divergent distance between the antapical horns. *Protoperidinium carum* is also similar to *P. paraoblongum* Sarai, Yamaguchi, Kawami, and Matsuoka, 2013 due to its anterior intercalary plate patterns. However, it can be distinguished by the cell-size, the shape of the sulcus, antapical horns, and dorsal-ventrally compressed cell (see in Phan-Tan et al., (2017), p. 142, fig. 9a–9f) [24].

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