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***Neoceratium* gen. nov., a New Genus for All Marine Species Currently Assigned to *Ceratium* (Dinophyceae)**

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Running title: *Neoceratium*, new genus for the marine *Ceratium*

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The dinoflagellate genus *Ceratium* contains marine and freshwater species. Freshwater species possess six cingular plates, thick plates in the concave ventral area and usually develop a third hypothecal horn. The marine *Ceratium* (>62 species) possess five cingular plates and thin plates in the concave ventral area; a third hypothecal horn is atypical. Resting cysts, a common feature in the freshwater species, are unreported in marine species. We illustrate for the first time resting cysts in marine *Ceratium* (*C. furca* and *C. candelabrum*). We obtained small subunit ribosomal RNA gene (SSU rDNA) sequences of 23 *Ceratium* species (more than one third of the total marine species described so far), with representatives of the four acknowledged subgenera. Phylogenetic analyses including the type species, the freshwater *C. hirundinella*, showed that the four available sequences of freshwater species formed a strongly supported subclade, very distant from the marine cluster. Our data support the splitting of *Ceratium* sensu lato into two genera. *Ceratium* sensu stricto should remain for freshwater species possessing six cingular plates (three cingular plates in dorsal view). The new genus name, *Neoceratium* gen. nov. should be applied to the marine species of *Ceratium* sensu lato that possess five cingular plates (two cingular plates in dorsal view).

Key words: alveolate evolution; Dinoflagellata; *Neoceratium* gen. nov.; new combination; resting cyst; SSU rDNA phylogeny.

Abbreviations: PCR, Polymerase chain reaction; SSU, small subunit; GPP complex (Gymnodinales, Peridinales, Prorocentrales) complex; rDNA, ribosomal deoxyribonucleic acid.

Introduction

The genus *Ceratium* Schrank is the earliest genus name in use for a dinoflagellate (Schrank 1793) and is one of the most important phytoplankton components. The large cell size of *Ceratium* species, mainly 100-300 μm long, make them easy to collect, while the tough theca ensures that morphological features essential for taxonomic analysis are preserved. The genus contains a large number of species that are truly planktonic and widely distributed in all seas and epicontinental waters. More than 120 marine species and infraspecies, varieties or forms, have been described and about 62 out of them are considered valid (Gómez 2005). Only four species, with numerous varieties, have colonized epicontinental waters (Popovsky and Pfister 1990).

The first illustrations of *Ceratium* appeared in the classic work of O.F. Müller (1786) that described one freshwater and one marine species as *Bursaria hirundinella* O.F. Müller and *Cercaria tripos* O.F. Müller, respectively. Under these genera, O.F. Müller described also other organisms that were not related to dinoflagellates (Müller 1786). Schrank (1793, 1802) created the genus *Ceratium* for the species *C. pleuroceras*, *C. tetraceras* and later *C. macroceras* from material collected in a lake in central Europe. Nitzsch (1817) realized that *Cercaria tripos* O.F. Müller belonged to Schrank's genus and made the transfer into *Ceratium*. Marine and freshwater species of *Ceratium* described thereafter were placed under Schrank's genus. *Bursaria hirundinella* O.F. Müller was also transferred into *Ceratium* (Dujardin 1841). The specimens described by Schrank (1793) - i.e. *C. pleuroceras*, *C. tetraceras* and *C. macroceras* - corresponded to *Bursaria hirundinella* and consequently they are regarded as synonyms of *C. hirundinella* (O.F. Müller) Dujardin (Popovsky and Pfister 1990). However, the name of the type species of the genus *Ceratium* has to be designed from the Schrank's species epithet: *C. pleuroceras* or *C. tetraceras*. Loeblich Jr and Loeblich III (1966) considered *C. pleuroceras*, the first of the species mentioned by Schrank (1793), as the type species of the genus. However, the International Code of Botanical Nomenclature (Farr et al. 1979) gave the priority to the lectotypification of *C. tetraceras*, the second species cited by Schrank, by Dujardin (1841).

Vanhöffen (1896) questioned the unity of the genus *Ceratium* and proposed its split into four genera based on the shape of the cell body and horns: *Amphiceratium*, *Biceratium*, *Proroceratium*, and *Ceratium* sensu stricto. Kofoid (1907a) studied the tabulation of seventeen marine species with representatives of each subgenus. He demonstrated the unity of the tabulation within the genus and rejected the split of *Ceratium* into four genera proposed by

Vanhöffen. This drove further studies of *Ceratium* towards the analysis of the silhouettes and ornamentation more than the tabulation as a criterion for species diagnosis. Vanhöffen's genera were ranked as subgenera: *Amphiceratium* (Vanhöffen) Ostenfeld (Ostenfeld 1903; Paulsen 1908), *Biceratium* (Vanhöffen) Ostenfeld (Ostenfeld 1903; Paulsen 1908), *Archaeoceratium* Jørgensen ((Jørgensen 1911) =*Poroceratium* (Vanhöffen) Pavillard (Pavillard 1916)) and *Ceratium* sensu stricto was named *Tripoceratium* Kofoid (Kofoid 1909, =*Euceratium* (Gran) Ostenfeld (Gran 1902; Ostenfeld 1903), =*Orthoceratium* Sournia (Sournia 1968)). Each subgenus was further divided into sections and subsections based on the cell contour similarities (Böhm 1931; Gran 1902; Jørgensen 1911, 1920; Karsten 1907; Kofoid 1909; Ostenfeld 1903; Paulsen 1908; Pavillard 1907). The freshwater species of *Ceratium* (section Cornuta) were placed in the subgenus *Biceratium* (Jørgensen 1911; Schiller 1937; Sournia 1968).

The subgenus *Tripoceratium* exhibits the classical 'anchor' shape in which both antapical horns proceed laterally after leaving the body (e.g. *C. tripos*). *Biceratium* includes species characterized by a distinct apical horn and unequal antapical horns that are directed posteriorly (e.g. *C. furca*). *Archaeoceratium* was created for a few rare species that have a high flattened apical horn (e.g. *C. gravidum*). The subgenus *Amphiceratium* (contains a few elongated species with both hypothecal horns directed posteriorly. The right horn is greatly reduced and the left one is often very extended (e.g. *C. fusus*).

The morphology of the freshwater and marine *Ceratium* has been investigated in detail with optical (Bourrelly 1968; Entz 1927), transmission and scanning electron microscopy (Dodge and Crawford 1970; Wetherbee 1975). In ancient literature the nomenclature of the thecal plates varies among the authors, until Kofoid (1907a) proposed a new system of tabulation that was widely accepted. After the analysis of seventeen marine species, Kofoid (1907a) concluded that *Ceratium* have four apical (4'), four precingular (4''), four cingular (4c), five postcingular (5''') and two antapical (2''') plates. Kofoid (1907a) did not investigate the plates of the concave ventral area that are highly delicate in the marine species and he erroneously considered four precingular plates (4'') instead of five (5''). The plates of the concave ventral area are more robust in the freshwater species and Entz (1927, p. 350) illustrated that these area was composed of three large plates (X, Y, Z) plus several sulcal platelets. He considered the plate X as the sixth precingular plate. Entz (1927) did not illustrate the cingular plates. Bourrelly (1968) examined three freshwater species and observed five cingular plates, whereas the marine species had only four (Kofoid had not examined any freshwater species when he proposed the tabulational unity of *Ceratium* in

1907). The dinoflagellate cingular and hypothecal plates are considered more conservative and stable taxonomical characters than those of the epitheca (Balech 1980). Nevertheless, the split of the genus based on the different number of cingular plates in marine and freshwater species has been a matter of debate for a long time (Sournia 1984). The plate formula was established as 4', 5'', 5''', 2'''' with 4c for the marine and 5c for freshwater species (Balech 1988), but the delicate plates of the concave ventral area were not considered. Wall and Evitt (1975) considered the plates X, Y and Z sensu Entz (1927) to be part of the precingular, cingular and postcingular series of plates, and they were named 6'', 6c and 6''', respectively (Wall and Evitt 1975). They also proposed one antapical and one posterior intercalary plate instead of two antapical plates, but this interpretation was not accepted in further literature (Balech 1988; Steidinger and Tangen 1997; Temponeras et al. 2000) and the nowadays accepted plate formula for the genus *Ceratium* is 4', 6'', 5 or 6c, 6''', 2'''' , plus 2 or more sulcal platelets (Steidinger and Tangen 1997). Consequently the marine species of *Ceratium* have five cingular plates, whereas the freshwater species possess six (Wall and Evitt 1975).

The development of molecular phylogeny has provided new tools for clarifying evolutionary relationships between protist species, the SSU rDNA being the most widely used marker for such studies (e.g., López-García et al. 2001; Saldarriaga et al. 2004; Saunders et al. 1997). *Ceratium* has received little attention despite its ecological importance. In May 2009, the only SSU rDNA sequences available on Genbank of this genus belonged to the freshwater *C. hirundinella* and the marine *C. furca*, *C. fusus*, *C. longipes* and *C. tenue*. In several phylogenetic trees, the marine and freshwater species of *Ceratium* clustered into separate clades (Duff et al. 2008; Logares et al. 2007; Saldarriaga et al. 2004).

In this study, we present 27 new sequences of marine species of *Ceratium*, with representatives of the four subgenera. Eleven of the 27 sequences corresponded to specimens collected in the type locality (the location where a species was originally described) of their respective species. This is of special interest to assure the species identity. We produced SSU rDNA phylogeny of the genus *Ceratium* and obtained strong support for the separation of freshwater and marine species in two different genera. In addition to the phylogeny results, our observations allowed us discussing the validity of some morphological characters that are used for the diagnostic separation between marine and freshwater species. For example, although resting cysts are common in freshwater species but were unknown from marine ones, we report their existence in at least two marine species (*C. furca* and *C. candelabrum*) Based on the above results, we propose a revision of the taxonomy of the genus *Ceratium* Schrank.

Results

We carried out a molecular phylogenetic study of specimens belonging to 23 species representing the four marine subgenera presently included in the genus *Ceratium*. The representative species of the subgenus *Tripoceratium* were *Ceratium arietinum*, *C. azoricum*, *C. concilians*, *C. contrarium*, *C. declinatum*, *C. euarquatum*, *C. hexacanthum*, *C. horridum*, *C. limulus*, *C. massiliense*, *C. paradoxoides*, *C. petersii*, *C. platycorne* and *C. symmetricum* (Fig. 1A-O). The sequences of *C. contrarium*, *C. euarquatum*, *C. hexacanthum*, *C. limulus*, *C. massiliense* and *C. symmetricum* were obtained from specimens collected in their type locality, the coastal waters of Marseille (France, Mediterranean Sea). The representative species of the subgenus *Biceratium* were *C. candelabrum*, *C. furca*, *C. kofoidii*, *C. minutum* and *C. pentagonum*, the latter collected from its type locality (Fig. 2A-E). The representative species of the subgenus *Amphiceratium* were its type, *C. fusus*, and *C. extensum*, collected from the type locality (Fig. 2F-H). The representative species of the subgenus *Archaeceratium* were the type species, *C. gravidum*, from its type locality (Fig. 2I) and *C. digitatum* (Fig. 2J-L).

To check the validity of the tabulation pattern as a criterion to differentiate between freshwater and marine *Ceratium*, we examined the plate arrangement of the freshwater type species of the genus, *C. hirundinella*, and twenty-two marine species: *Ceratium arietinum*, *C. azoricum*, *C. candelabrum*, *C. concilians*, *C. declinatum*, *C. furca*, *C. fusus*, *C. gibberum*, *C. hexacanthum*, *C. horridum*, *C. limulus*, *C. macroceros*, *C. massiliense*, *C. minutum*, *C. paradoxoides*, *C. pentagonum*, *C. petersii*, *C. platycorne*, *C. pulchellum*, *C. ranipes*, *C. symmetricum* and *C. tripos* (Fig. 3A-L). In the freshwater species, *C. hirundinella*, the first and fifth cingular plates are short and ventral (Fig. 3A). The concave ventral area composed of three large plates and small sulcal platelets. The sixth precingular plate (6'') is the largest of the concave ventral area, and two smaller plates, the sixth cingular (6c) and six postcingular (6''') are located posteriorly (Fig. 3B). On the left side of these three plates, there are the sulcal plates, but their number and position could not be determined. In dorsal view, the cingulum showed two sutures, in line with the suture of the precingular plates 2''/3'' and 3''/4''. The second cingular plate is longer than the third and fourth ones (Fig. 3C-D). In marine species, the first and fourth cingular plates are short and located in the ventral portion of the cingulum (Fig. 3E). The second and third cingular plates are long and located in the dorsal portion of the cingulum (Fig. 3G, I). The suture between the plate 2c and 3c is at

the middle of the cingulum and is in line with the sutures of the precingular plates 2''/3'' and postcingular plates 3'''/4''' (Fig. 3F, H). The first postcingular plate is larger in the freshwater species than in the marine species. In the marine species, the plates of the concave ventral area are hyaline and thinner than those of the freshwater species. We hardly tried to dissect these plates, but were unsuccessful with the exception of one specimen of *C. macroceros* that clearly showed the sixth precingular plate (6'') attached to the epicone. This highly hyaline plate was covered with fairly regularly spaced pores. At the left side of this plate, a long, narrow and smooth plate - most probably belonging to the sulcal series - was visible (Fig. 3K). In live specimens, at least two narrow sulcal plates appeared on the right side of the canal that harbors the longitudinal flagellum (Fig. 3J). According to the interpretation proposed by Wall and Evitt (1975) that included the plates of the concave ventral area, *Ceratium hirundinella* and the marine species have six and five cingular plates, respectively (Fig. 3A-L).

We obtained 27 new SSU rDNA sequences from 23 marine species of the genus *Ceratium*, from the isolation of single cells that were identified and photographed in light microscopy (Table 1). Replicate specimens of *C. candelabrum*, *C. concilians*, *C. limulus* and *C. petersii* collected during different periods provided very similar (>99% identity) but not identical sequences, suggesting the existence of some intraspecific polymorphism for this gene.

The phylogenetic position of the *Ceratium* species was analyzed by means of a global alignment of 65 SSU rDNA sequences representing various taxa including armoured and unarmoured dinoflagellates, especially gonyaulacoid species, with *Scrippsiella nutricula* (Brandt) Banaszak *et* Iglesias, *Symbiodinium microadriaticum* Freudenthal and *Polarella glacialis* Montresor, Procaccini *et* Stoecker as outgroup taxa (Fig. 4). Our SSU rDNA phylogeny strongly supports (posterior probability, PP, of 1) a clade composed of freshwater and marine *Ceratium* species. They are placed as a part of the so-called GPP complex sensu Saunders *et al.* (1997), composed of species of the orders Gymnodiniales, Peridinales, Procentrales, and Blastodinales. The monophyly of the freshwater and marine *Ceratium* also supports the classification of *Ceratium* as the only member of the Ceratiaceae. In all analyses, the four freshwater *Ceratium* SSU rDNA sequences formed a strongly supported subclade, distantly related to the marine cluster (Fig. 4). SSU rDNA sequences from marine *Ceratium* species were highly similar for this phylogenetic marker (Fig. 5). The greatest difference between the marine species in the 1078 bp of SSU rDNA sequence used in our analyses was of only 22 substitutions (*Ceratium gravidum* vs. *C. furca*), with an average of 7 substitutions (0.65%). By contrast, the marine and freshwater *Ceratium* sequences differed in

a range between 91 and 105 substitutions, with an average of 95 substitutions (8.81%). The sequences of the closest relatives to *Ceratium*, *Ceratocorys horrida* Stein and *Protoceratium reticulatum* (Claparède et Lachmann) Bütschli, differed in average by 82 and 103 substitutions (7.6% and 9.62%, respectively) from the marine and freshwater *Ceratium* sequences, respectively. Taking into account the large evolutionary distance and the different number of cingular plates between the freshwater and marine *Ceratium* species, we propose their separations and the erection of a new genus, *Neoceratium*, for the marine species.

During this study, we observed structures interpreted as resting cysts of *Ceratium furca* and *C. candelabrum* (Fig. 6A-E) cells from natural samples. These cysts were obovate in shape, dark brown and surrounded by a thick wall. Although exceedingly rare, we observed the formation of resting cysts in a senescent population of *C. candelabrum* (Fig. 6A-B) collected at the pier of the Station Marine of Endoume, Marseille. We also observed up to five resting cysts in a sample of *Ceratium furca* collected from the shallow coastal Berre Lagoon (43°30'N, 5°10'E) (Fig. 6C-E). These findings call for the reconsideration of this trait, previously thought to be exclusive of freshwater species, as diagnostic character. Nevertheless, further studies are required to definitely prove the resting character of these stages.

Taxonomic description

Alveolata Cavalier-Smith 1991

Dinophyceae G.S. West et Fritsch 1927

Gonyaulacales F.J.R. Taylor 1980

Ceratiaceae Willey et Hickson 1909

Neoceratium F. Gómez, D. Moreira et P. López-García, *gen. nov.*

Diagnosis: *Dinoflagellata thecata marina in directione dorsoventrali compressa, area ventralis depressa; corniculis maxime tribus. Gonyaulacales duabus tabulis cinguli a dorso visis. Tabulatione tabularum 4', 6'', 5c, 6''', 2'''' et tabulis sulci.*

Marine thecate dinoflagellate dorso-ventrally compressed, depressed ventral area and up to three horns. Gonyaulacaleans with two cingular plates in dorsal view. Plate tabulation: 4', 6'', 5c, 6''', 2'''' and sulcal platelets.

Type species: Neoceratium furca (Ehrenberg) F. Gómez, D. Moreira et P. López-García comb. nov. (designated here).

Etymology: Greek *neo-*, new; Greek *keration-* little horn (neuter).

Basionym: *Peridinium furca* Ehrenberg (1834, p. 574, plate II, fig. 2).

Nomenclatural synonyms: *Ceratium furca* (Ehrenberg) Claparède et Lachmann, *Ceratophorus furca* (Ehrenberg) Diesing, *Biceratium furca* Vanhöffen.

Taxonomical synonyms: *Peridinium eugrammum* Ehrenberg

Other species belonging to *Neoceratium*: See Table 2.

Discussion

Our data strongly support the separation of the marine and freshwater species of *Ceratium* into two distinct genera based on both the number of cingular plates and the considerable evolutionary distance of their respective SSU rDNA sequences, which form two well-defined monophyletic clades. The erection of a separate genus for the marine species of *Ceratium*, as occurred for *Peridinium* Ehrenberg / *Protoperidinium* Bergh, is a matter of debate (Sournia 1984). Until the 1970's, *Peridinium* sensu lato was the most speciose dinoflagellate genus, with more than 500 species, including both marine and freshwater species. It was noted that the marine *Peridinium* possess four cingular plates, whereas the freshwater *Peridinium* possess five or six. The marine species were transferred into *Protoperidinium* (Balech 1974), and currently *Peridinium* sensu stricto and *Protoperidinium* are placed into separate families (Balech 1988).

Our results show that the freshwater species of *Ceratium* are not very closely related in phylogenetic trees to the members of the subgenus *Biceratium* (type, *N. furca*), despite their original placement in the classic taxonomic scheme (Jørgensen 1911; Schiller 1937; Sournia 1968). Indeed, the subgeneric separation based on silhouettes or ornamentation is not supported by our SSU rDNA phylogenies, which showed the marine species of the four subgenera appear intermixed, with very few differences in the SSU rDNA sequences (Figs. 4 and 5). The type species of the genus *Ceratium* is a freshwater species, and the marine *Ceratium* species should therefore be placed in one or several separate genera. In order to make the taxonomy of these species compatible with phylogenetic data, the following options are available: i) to reinstate any of the valid and legitimate generic taxonomic juniors (*Amphiceratium*, *Biceratium*, *Poroceratium*); ii) to rank any of the subgenera (*Archaeoceratium*, *Euceratium*, *Macroceratium*, *Orthoceratium*, *Tripoceratium*) at the genus

level; or iii) to establish a new genus name for current marine *Ceratium*. To avoid potential confusion derived from the use of previous subgeneric names, we propose a new generic name, *Neoceratium*, which has not been previously used in the botanical nomenclature, to encompass the marine species. The type species of this new genus, *Neoceratium furca*, is the most ubiquitous and common marine species in coastal and oceanic waters.

Our observations support that the number of cingular plates is the most robust morphological character for separating the genera *Neoceratium* and *Ceratium* sensu stricto. These differences in the tabulation were already illustrated in other studies (Fig. 7A-O; Bourrelly 1968; Graham 1942; Kofoed 1907; Schiller 1937). The number of precingular, postcingular and antapical plates of both genera are similar to those of the numerous Gonyaulacales (*Goniodoma* Stein, *Gonyaulax* Diesing, *Protoceratium* Bergh, *Spiraulax* Kofoed, *Pyrodinium* Plate and others). The interpretation by Wall and Evitt (1975) of one antapical plate and one posterior intercalary plate instead of two antapical plates have not been accepted in further literature (Balech 1988; Steidinger and Tangen 1997; Temponeras et al. 2000). The number of apical or anterior intercalary plates is more variable among the Gonyaulacales (Steidinger and Tangen 1997). Near all the Gonyaulacales have six cingular plates and only a few genera have more (*Fragilidinium* Balech, *Pyrophacus* Stein). *Neoceratium* have exceptionally five cingular plates. This suggests that hypothetically *Neoceratium* derived from a gonyaulacean ancestor with six cingular plates in that the third and fourth cingular plates fused into a single plate.

The plates of the concave ventral area of *Neoceratium* are rarely observed because they are hyaline and delicate when compared with the other plates (Fig. 3J-K). Very few studies have partially illustrated these plates (Graham 1942; Steidinger and Williams 1970; Fig. 7N). Chloroplast-containing Gonyaulacales are able to feed on prey cells by engulfing them through the apical horn and through the sulcal area (Jeong et al. 2005). *Neoceratium* species ingest the preys through the sulcal area (Jacobson 1999), suggesting that the sulcal plates may be moveable, deformable or re-synthesized after each prey ingestion. The difficulty to observe these hyaline structures, precludes using them as diagnostic criteria.

In contrast with the robustness of the tabulation pattern, especially from the cingulum and hypotheca, the length of the horns can vary intraspecifically in both marine and freshwater species, being absent or vestigial in numerous species (Meave del Castillo et al. 2003; Sournia 1968). The horn orientation or shape is also a poor taxonomical criterion for generic separation. The formation of a fourth horn (a third hypothecal horn) is a common feature of the freshwater species that typically show strong intraspecific morphological variability

(Popovski and Pfiester 1990; Schiller 1937). The third hypothecal horn is also known from the marine species, but this is a rare phenomenon often observed in cultures and only sporadically in samples from natural waters (Hasle and Nordli 1951; Steidinger and Williams 1970).

The two genera *Ceratium* sensu stricto and *Neoceratium* also differ in their habitats: freshwater the first and marine the second, as occurred in the case of *Peridinium* and *Protoperidinium*. This agrees with the observations made by Logares et al. (2007), who based on extensive 18S-28S rDNA dinoflagellate phylogenies, concluded that marine and freshwater species are usually not closely related and most of the marine-freshwater transitions do not seem to have occurred recently.

Previous authors have reported that the formation of resting cysts is unknown in marine *Ceratium* sensu lato (Sournia 1986), whereas it is a common feature of the freshwater species. In the epicontinental waters, the populations of *Ceratium* are subjected to strong environmental changes including, often, desiccation. The resting cysts constitute a strategy to survive unfavorable conditions or for airborne dispersal. In open marine environments, by contrast, extreme environmental conditions such as desiccation are more improbable. The cysts of *Neoceratium* observed in this study (Fig. 6C-E) coincided with a senescent population of *C. furca* heavily predated by the phagotrophic dinoflagellate *Polykrikos kofoidii* Chatton. Although the marine *Ceratium* have chloroplasts, mixotrophy is a common feature (Jacobson 1999). Consequently, the populations of *Ceratium* may be better adapted to survive in conditions of nutrient depletion, when compared to other gonyaulacoid phototrophic dinoflagellates. This may explain why the formation of resting cysts is so rarely observed in *Neoceratium*.

Although resting cysts can no longer be considered an exclusive feature of the freshwater *Ceratium*, the number of cingular plates, on the contrary, appears a stable taxonomical character for the generic separation of thecate dinoflagellates (Balech 1980). We conclude that the separation of marine and freshwater species of *Ceratium* is supported by both morphological and molecular data.

Methods

Sampling and isolation: Fifty of the 62 marine *Ceratium* species have been recorded in the Mediterranean Sea (Gómez 2003). The Gulf of Lions (France) is the type locality of 19 species; more than one fourth of the total marine species, and sixteen out of them were first

described in samples from the coast of Marseille (Gourret 1883). In this study, the organisms were collected by slowly filtering surface seawater taken from the end of the pier (depth 3 m) of the Station Marine d'Endoume, Marseille (43°16'48"N, 5°20'57"E) from October 2007 to September 2008. A strainer with netting of 20, 40 or 60- μ m mesh-size was used to collect the organisms and the filtered volume varied between 10 and 100 liters, according to the concentration of particles. The procedure was repeated 3-7 times per day. The concentrated sample was examined in Utermöhl chambers at 100 \times magnification with a Nikon inverted microscope (Nikon Eclipse TE200) and was photographed at 200 \times or 400 \times magnification with a digital camera (Nikon Coolpix E995). Sampling continued from October 2008 to May 2009 in the surface waters of the port (depth of 2 m) of Banyuls sur Mer, France (42°28'50"N, 3°08'09"E). The samples were prepared with the same procedure above described. The specimens were observed with an Olympus inverted microscope (Olympus IX51) and photographed with an Olympus DP71 digital camera. In all cases, each specimen was individually micropipetted with a fine capillary into a clean chamber and washed several times in serial drops of 0.2- μ m filtered and sterilized seawater. Finally, the specimen was deposited into a 1.5 ml Eppendorf tube filled with several drops of 100% ethanol. The sample was kept at room temperature and in darkness until the molecular analysis could be performed. Each PCR sample contained between 1 and 5 specimens of identical morphology collected from the same sample.

Observations of the thecal plate pattern: Specimens of *Neoceratium* were collected from the port of Banyuls sur Mer in February, March and April of 2009. *Ceratium hirundinella* was collected from the Spanish reservoir of Guadalest (0°12'02"N, 38°41'05"W) on February 17th, 2009. The specimens were fixed with glutaraldehyde and treated with 2% sodium hypochlorite solution for the plate dissection. Other specimens were stained with 0.01% Fluorescent Brightener 28 (Sigma-Aldrich, France). They were observed using an Olympus inverted fluorescence microscope (IX51) and photographed with a digital camera (DP71 Olympus).

PCR amplification of small subunit rRNA genes (SSU rDNAs) and sequencing: The specimens fixed in ethanol were centrifuged gently for 5 minutes at 3,000 rpm. Ethanol was then evaporated in a vacuum desiccator and the specimens resuspended directly in, depending on the amount of cells, 25 to 50 μ l of Ex TaKaRa (TaKaRa, distributed by Lonza Cia., Levallois-Perret, France) PCR reaction mix containing 10 to 20 pmol of the eukaryotic-

specific SSU rDNA primers EK-42F (5'-CTCAARGAYTAAGCCATGCA-3') and EK-1520R (5'-CYGCAGGTTACCTAC-3'). The PCR reactions were performed under the following conditions: 2 min denaturation at 94 °C; 10 cycles of 'touch-down' PCR (denaturation at 94 °C for 15 s; a 30-s annealing step at decreasing temperature from 65 down to 55 °C employing a 1 °C decrease with each cycle, extension at 72 °C for 2 min); 20 additional cycles with 55 °C of annealing temperature; and a final elongation step of 7 min at 72 °C. A nested PCR reaction was then carried out using 2 to 5 µl of the first PCR reaction in a GoTaq (Promega, Lyon, France) polymerase reaction mix containing the eukaryotic-specific primers EK-82F (5'-GAAACTGCGAATGGCTC-3') and EK-1498R (5'-CACCTACGGAAACCTTGTTA-3') and similar PCR conditions as above except for an increase in the total number of cycles from 30 to 35 or 50. PCR products were checked by agarose gel electrophoresis. PCR products yielding a band of expected size were retained for further sequencing with EK-82F. In all cases, a third, semi-nested PCR was carried out using the dinoflagellate specific primer DIN464F (5'-TAACAATACAGGGCATCCAT-3') (Gómez et al. 2009). We used 0.3 to 3 µl of the second PCR reaction as template for the third PCR amplification reaction, which was carried out under identical conditions to the precedent nested PCR reaction. Amplicons of expected size were then sequenced bidirectionally using DIN464F and EK-1498R (Cogenics, Meylan, France). In a few cases, near full-length SSU rDNAs were obtained by sequencing amplicons from the second PCR reaction with EK-82F. However, in many cases this was prevented by the amplification of a mixture of eukaryotic SSU rDNAs, presumably from ingested prey.

Other sequences: Additional SSU rDNA sequences of *Ceratium* spp. were downloaded from GenBank (Table 1). Four sequences corresponded to freshwater *Ceratium*. Among them, the sequence of *C. hirundinella* collected from a lake in the Alps (Auinger et al. 2008) is the closest to the type locality (Schrank 1793). *Ceratium tenue* is considered a synonym of *C. horridum* by some authors (Gómez 2005; Sournia 1968) and a separate species by others (Balech 1988).

Phylogenetic analyses: The new sequences were aligned to a large multiple sequence alignment containing 890 publicly available complete or nearly complete dinoflagellate SSU rDNA sequences using the profile alignment option of MUSCLE 3.7 (Edgar 2004). The resulting alignment was manually inspected using the program ED of the MUST package (Philippe 1993). Ambiguously aligned regions and gaps were excluded from phylogenetic

analyses. Preliminary phylogenetic trees with all sequences were constructed using the Neighbour Joining (NJ) method (Saitou and Nei 1987) implemented in the MUST package (Philippe 1993). These trees allowed the identification of the closest relatives of our sequences, which were selected, together with a sample of other dinoflagellate species, to carry out more computationally-intensive Maximum Likelihood (ML) and Bayesian Inference (BI) analyses. ML analyses were conducted with the program TREEFINDER (Jobb et al. 2004) by applying a GTR + Γ + I model of nucleotide substitution, taking into account a proportion of invariable sites, and a Γ -shaped distribution of substitution rates with four rate categories. BI analyses were carried out with the program PHYLOBAYES through the application of a GTR + CAT Bayesian mixture model (Lartillot and Philippe 2004). Sequences were deposited in GenBank with accession numbers FJ402942-FJ402966 and FJ824910-FJ824911 (see Table 1). Sequence alignments are available from Treebase under accession number SN4515.

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Table 1. List of *Ceratium/Neoceratium* SSU rDNA sequences used for the phylogenetic analysis. The species names, strain codes and literature references are listed as they appear in GenBank. Accession numbers, geographical origin of the strains and isolation dates, between parentheses, are also provided.

Taxa	GenBank No	Geographical origin	Reference
<i>Ceratium furca</i> (= <i>N. furca</i>)	AJ276699	Hulu Island, China	Zhuang et al. unpubl.
<i>C. fusus</i> CCMP154 (= <i>N. fusus</i>)	AF022153	West Boothbay Harbor, ME, U.S.A.	Saunders et al. (1997)
<i>C. hirundinella</i> WA28-13	EU025021	Lake in Mondsee, Austria	Auinger et al. (2008)
<i>C. hirundinella</i>	AY443014	Lake in San Juan Is., WA, U.S.A.	Saldarriaga et al. (2004)
<i>Ceratium</i> -like HZ-2004	AY460574	Mirror Lake, Storrs, CT, U.S.A.	Zhang et al. (2005)
<i>Ceratium</i> sp. HCB-2005	DQ487192	Garden Pond, Akron, OH, U.S.A.	Duff et al. (2008)
<i>C. longipes</i> CCMP 1770 (= <i>N. longipes</i>)	DQ388462	Long Island Sound, CT, U.S.A.	Lin et al. (2006)
<i>C. tenue</i> MUCC248 (= <i>N. tenue</i>)	AF022192	West Boothbay Harbor, ME, U.S.A.	Saunders et al. (1997)
<i>N. arietinum</i> FG27	FJ402956	Marseille, France (28 Mar 2008)	This study, Fig. 1A
<i>N. azoricum</i> FG28	FJ402954	Marseille, France (17 Mar 2008)	This study, Fig. 1L
<i>N. candelabrum</i> FG7	FJ402955	Marseille, France (22 Dec 2007)	This study, Fig. 2A
<i>N. candelabrum</i> FG29	FJ402945	Marseille, France (25 Mar 2008)	This study
<i>N. concilians</i> FG3	FJ402944	Marseille, France (7 Nov 2007)	This study, Fig. 1B
<i>N. concilians</i> FG30	FJ402950	Marseille, France (17 Mar 2008)	This study
<i>N. contrarium</i> FG67	FJ402959	Marseille, France (16 Apr 2008)	This study, Fig. 1C
<i>N. declinatum</i> FG8	FJ402949	Marseille, France (26 Dec 2007)	This study, Fig. 1D
<i>N. digitatum</i> FG231	FJ824910	Marseille, France (2 Sept 2008)	This study, Fig. 2J-L
<i>N. euarquatatum</i> FG32	FJ402946	Marseille, France (16 Apr 2008)	This study, Fig. 1E
<i>N. extensum</i> FG69	FJ402957	Marseille, France (12 Jun 2008)	This study, Fig. 2G-H

<i>N. furca</i> FG70b	FJ402966	Marseille, France (6 Jun 2008)	This study, Fig. 2B
<i>N. fusus</i> FG71	FJ402958	Marseille, France (8 May 2008)	This study, Fig. 2F
<i>N. gravidum</i> FG73	FJ402961	Marseille, France (14 Apr 2008)	This study, Fig. 2I
<i>N. hexacanthum</i> FG2	FJ402943	Marseille, France (23 Dec 2008)	This study, Fig. 1H-I
<i>N. horridum</i> FG72	FJ402960	Marseille, France (8 Apr 2008)	This study, Fig. 1G
<i>N. kofoidii</i> FG77	FJ402963	Marseille, France (16 Apr 2008)	This study, Fig. 2C
<i>N. limulus</i> FG35	FJ402952	Marseille, France (14 Mar 2008)	This study, Fig. 1N
<i>N. limulus</i> FG74	FJ402962	Marseille, France (12 Jun 2008)	This study
<i>N. massiliense</i> FG37	FJ402942	Marseille, France (11 Apr 2008)	This study, Fig. 1F
<i>N. minutum</i> FG79	FJ402964	Marseille, France (8 Apr 2008)	This study, Fig. 2D
<i>N. platycorne</i> FG261	FJ824911	Banyuls sur Mer, France (27 Nov 2008)	This study, Fig. 1K
<i>N. paradoxoides</i> FG80	FJ402965	Marseille, France (17 Apr 2008)	This study, Fig. 1O
<i>N. pentagonum</i> FG1	FJ402948	Marseille, France (7 Nov 2007)	This study, Fig. 2E
<i>N. petersii</i> FG5	FJ402951	Marseille, France (23 Dec 2007)	This study
<i>N. petersii</i> FG40	FJ402953	Marseille, France (19 Mar 2008)	This study, Fig. 1M
<i>N. symmetricum</i> FG41	FJ402947	Marseille, France (14 Mar 2008)	This study, Fig. 1J

Table 2. List of new combinations of *Neoceratium* F. Gómez, D. Moreira et P. López-García.

New combination of <i>Neoceratium</i>	Basionym	Synonyms	Reference
<i>N. arcticum</i> (Ehrenberg) F. Gómez, D. Moreira et P. López-García	<i>C. arcticum</i> (Ehrenberg) Cleve	<i>C. labradoricum</i> Vanhöffen	Ehrenberg (1853), 528, (1854) T. 35, A. fig. 24
<i>N. arietinum</i> (Cleve) F. Gómez, D. Moreira et P. López-García	<i>C. arietinum</i> Cleve	<i>C. bucephalum</i> (Cleve) Cleve	Cleve (1900), 13, T. 7, fig. 3
<i>N. aultii</i> (Graham et Bronikovsky) F. Gómez, D. Moreira et P. López-García	<i>C. aultii</i> Graham et Bronikovsky		Graham and Bronikovsky (1944), 30, fig. 15f-g
<i>N. axiale</i> (Kofoid) F. Gómez, D. Moreira et P. López-García	<i>C. axiale</i> Kofoid		Kofoid (1907b), 170, T. 4, fig. 26
<i>N. azoricum</i> (Cleve) F. Gómez, D. Moreira et P. López-García	<i>C. azoricum</i> Cleve		Cleve (1900), 13, T. 7, fig. 6, 7
<i>N. balechii</i> (Meave del Castillo),	<i>C. balechii</i>		Meave del Castillo et

Okolodkov <i>et</i> Zamudio) F. Gómez, D. Moreira <i>et</i> P. López-García	Meave del Castillo, Okolodkov <i>et</i> Zamudio		al. (2003), 83, fig. 11a-c
<i>N. belone</i> (Cleve) F. Gómez, D. Moreira <i>et</i> P. López-García	<i>C. belone</i> Cleve	<i>C. pacificum</i> Schröder	Cleve (1900), 3, T. 7, fig. 13
<i>N. biceps</i> (Claparède <i>et</i> Lachmann) F. Gómez, D. Moreira <i>et</i> P. López-García	<i>C. biceps</i> Claparède <i>et</i> Lachmann		Claparède and Lachmann (1859), 400, T. 19, fig. 8
<i>N. bigelowii</i> (Kofoid) F. Gómez, D. Moreira <i>et</i> P. López-García	<i>C. bigelowii</i> Kofoid		Kofoid (1907b), 170, T. 3, fig. 22
<i>N. breve</i> (Ostenfeld <i>et</i> Schmidt) F. Gómez, D. Moreira <i>et</i> P. López-García	<i>C. breve</i> (Ostenfeld <i>et</i> Schmidt) Schröder	<i>C. schmidtii</i> Jørgensen	Ostenfeld and Schmidt (1901), 164, fig. 13
<i>N. brunellii</i> (Rampi) F. Gómez, D. Moreira <i>et</i> P. López-García	<i>C. brunellii</i> Rampi		Rampi (1942), 222, fig. 1
<i>N. candelabrum</i> (Ehrenberg) F. Gómez, D. Moreira <i>et</i> P. López-García	<i>C. candelabrum</i> (Ehrenberg) Stein	<i>C. globatum</i> Gourret	Ehrenberg (1859), 792, (1873), 3, fig. 2, 3.
<i>N. carnegiei</i> (Graham <i>et</i> Bronikowsky) F. Gómez, D. Moreira <i>et</i> P. López-García	<i>C. carnegiei</i> Graham <i>et</i> Bronikowsky		Graham and Bronikowsky (1944), 34, fig. 18A-C
<i>N. carriense</i> (Gourret) F. Gómez, D. Moreira <i>et</i> P. López-García	<i>C. carriense</i> Gourret	<i>C. hundhausenii</i> Schröder	Gourret (1883), T. 4, fig. 57
<i>N. cephalotum</i> (Lemmermann) F. Gómez, D. Moreira <i>et</i> P. López-García	<i>C. cephalotum</i> (Lemmermann) Jørgensen	<i>C. gravidum</i> var. <i>cephalotum</i> Lemmermann	Lemmermann (1900), 349, T.1, fig. 15
<i>N. compressum</i> (Gran) F. Gómez, D. Moreira <i>et</i> P. López-García	<i>C. compressum</i> Gran		Gran (1902), 196, fig. 16
<i>N. concilians</i> (Jørgensen) F. Gómez, D. Moreira <i>et</i> P. López-García	<i>C. concilians</i> Jørgensen	<i>C. tripos</i> var. <i>contortum</i> Gourret	Jørgensen (1920), 72, fig. 69
<i>N. contortum</i> (Gourret) F. Gómez, D. Moreira <i>et</i> P. López-García	<i>C. contortum</i> Gourret	<i>C. gibberum</i> var. <i>contortum</i> Gourret	Gourret (1883), T. 2, fig. 33

<i>N. contrarium</i> (Gourret) F. Gómez, D. Moreira et P. López-García	<i>C. contrarium</i> (Gourret) Pavillard	<i>C. trichoceros</i> var. <i>contrarium</i> (Gourret) Schiller	Gourret (1883), 31, T. 2, fig. 36
<i>N. declinatum</i> (Karsten) F. Gómez, D. Moreira et P. López-García	<i>C. declinatum</i> (Karsten) Jørgensen	<i>C. tripos</i> <i>declinatum</i> Karsten	Karsten (1907), T. 48, fig. 2a,b
<i>N. deflexum</i> (Kofoid) F. Gómez, D. Moreira et P. López-García	<i>C. deflexum</i> (Kofoid) Jørgensen	<i>C. californiense</i> Karsten	Kofoid (1907b), 304, T. 24, fig. 13-15
<i>N. dens</i> (Ostenfeld et Schmidt) F. Gómez, D. Moreira et P. López-García	<i>C. dens</i> Ostenfeld et Schmidt		Ostenfeld and Schmidt (1901), 165, fig. 16
<i>N. digitatum</i> (Schütt) F. Gómez, D. Moreira et P. López-García	<i>C. digitatum</i> Schütt		Schütt (1895), T. 12, fig. 42
<i>N. divaricatum</i> (Lemmermann) F. Gómez, D. Moreira et P. López-García	<i>C. divaricatum</i> (Lemmermann) Kofoid	<i>C. tripos</i> var. <i>divaricatum</i> Lemmermann	Kofoid (1908), 380, fig. 31
<i>N. egyptiacum</i> (Halim) F. Gómez, D. Moreira et P. López-García	<i>C. egyptiacum</i> Halim		Halim (1963), 497, fig. 15-18
<i>N. euarquatatum</i> (Jørgensen) F. Gómez, D. Moreira et P. López-García	<i>C. euarquatatum</i> Jørgensen	<i>C. arcuatum</i> (Gourret) Pavillard	Gourret (1883), 25, T. 2, fig. 42
<i>N. extensum</i> (Gourret) F. Gómez, D. Moreira et P. López-García	<i>C. extensum</i> (Gourret) Cleve	<i>C. strictum</i> Kofoid	Gourret (1883), 49, T. 1, fig. 13
<i>N. falcatiforme</i> (Jørgensen) F. Gómez, D. Moreira et P. López-García	<i>C. falcatiforme</i> Jørgensen	<i>C. inflatum</i> <i>falcatiforme</i> Peters 1934)	Jørgensen (1920), 40, fig. 29
<i>N. falcatum</i> (Kofoid) F. Gómez, D. Moreira et P. López-García	<i>C. falcatum</i> (Kofoid) Jørgensen	<i>C. pennatum</i> f. <i>falcata</i> Kofoid	Kofoid (1907b), 172, T. 2, fig. 14
<i>N. filicorne</i> (Stemann Nielsen) F. Gómez, D. Moreira et P. López-García	<i>C. filicorne</i> Steemann Nielsen		Stemann Nielsen (1934), 19, fig. 39
<i>N. furca</i> (Ehrenberg) F. Gómez, D. Moreira et P. López-García	<i>C. furca</i> (Ehrenberg)		Ehrenberg (1833), 270 (1838), 256, T.

	Claparède <i>et</i> Lachmann		22, fig. 21
<i>N. fusus</i> (Ehrenberg) F. Gómez, D. Moreira <i>et</i> P. López-García	<i>C. fusus</i> (Ehrenberg) Dujardin	<i>C. seta</i> (Ehrenberg) Jørgensen	Ehrenberg (1833), 271 (1834), 246, T. 22, fig. 20
<i>N. geniculatum</i> (Lemmermann) F. Gómez, D. Moreira <i>et</i> P. López-García	<i>C. geniculatum</i> (Lemmermann) Cleve	<i>C. tricarinatum</i> Kofoid	Lemmermann (1900), T. 1, fig. 17
<i>N. gibberum</i> (Gourret) F. Gómez, D. Moreira <i>et</i> P. López-García	<i>C. gibberum</i> Gourret		Gourret (1883), 36, T. 2, fig. 35
<i>N. gravidum</i> (Gourret) F. Gómez, D. Moreira <i>et</i> P. López-García	<i>C. gravidum</i> Gourret	<i>C. oviforme</i> Daday	Gourret (1883), 58, T. 1, fig. 15
<i>N. hexacanthum</i> (Gourret) F. Gómez, D. Moreira <i>et</i> P. López-García	<i>C. hexacanthum</i> Gourret	<i>C. reticulatum</i> (Pouchet) Cleve	Gourret (1883), 36, T. 3, fig. 49
<i>N. hircus</i> (Schröder) F. Gómez, D. Moreira <i>et</i> P. López-García	<i>C. hircus</i> Schröder	<i>C. furca</i> var. <i>hircus</i> (Schröder) Margalef	Schröder (1909), 211, fig. 2a-d
<i>N. horridum</i> (Gran) F. Gómez, D. Moreira <i>et</i> P. López-García	<i>C. horridum</i> Gran	<i>C. intermedium</i> (Jørgensen) Jørgensen, <i>C. claviger</i> Kofoid	Gran (1902), 54, 193, 194
<i>N. humile</i> (Jørgensen) F. Gómez, D. Moreira <i>et</i> P. López-García	<i>C. humile</i> Jørgensen		Jørgensen (1911), 40, fig. 82, 83
<i>N. incisum</i> (Karsten) F. Gómez, D. Moreira <i>et</i> P. López-García	<i>C. incisum</i> (Karsten) Jørgensen		Karsten (1906), T. 23, fig. 6a,b
<i>N. inflatum</i> (Kofoid) F. Gómez, D. Moreira <i>et</i> P. López-García	<i>C. inflatum</i> (Kofoid) Jørgensen	<i>C. nipponicum</i> Okamura	Kofoid (1907b), T. 2, fig. 13
<i>N. karstenii</i> (Pavillard) F. Gómez, D. Moreira <i>et</i> P. López-García	<i>C. karstenii</i> Pavillard		Pavillard (1907), 152
<i>N. kofoidii</i> (Jørgensen) F. Gómez, D. Moreira <i>et</i> P. López-García	<i>C. kofoidii</i> Jørgensen	<i>C. boehmii</i> Graham <i>et</i> Bronnikovsky	Jørgensen (1911), 23, fig. 38, 39

			1944)
<i>N. lanceolatum</i> (Kofoid) F. Gómez, D. Moreira et P. López-García	<i>C. lanceolatum</i> Kofoid		Kofoid (1907b), 172, T. 3, fig. 17
<i>N. limulus</i> (Gourret) F. Gómez, D. Moreira et P. López-García	<i>C. limulus</i> Gourret		Gourret (1883), 33, T. 1, fig. 7
<i>N. lineatum</i> (Ehrenberg) F. Gómez, D. Moreira et P. López-García	<i>C. lineatum</i> (Ehrenberg) Cleve		Ehrenberg (1854), T. 25C, (1854), 2, 240
<i>N. longipes</i> (Bailey) F. Gómez, D. Moreira et P. López-García	<i>C. longipes</i> (Bailey) Gran		Bailey (1855), 12, fig. 35
<i>N. longirostrum</i> (Gourret) F. Gómez, D. Moreira et P. López-García	<i>C. longirostrum</i> Gourret	<i>C. scapiforme</i> Kofoid	Gourret (1883), 55, T. 4, fig. 65
<i>N. longissimum</i> (Schröder) F. Gómez, D. Moreira et P. López-García	<i>C. longissimum</i> (Schröder) Kofoid		Schröder (1900), 16, T. 1, fig. 171
<i>N. lunula</i> (Schimper ex Karsten) F. Gómez, D. Moreira et P. López-García	<i>C. lunula</i> (Schimper ex Karsten) Jørgensen		Schimper in Chun (1900), 73, fig. A
<i>N. macroceros</i> (Ehrenberg) F. Gómez, D. Moreira et P. López-García	<i>C. macroceros</i> (Ehrenberg) Vanhöffen		Ehrenberg (1840), 201
<i>N. massiliense</i> (Gourret) F. Gómez, D. Moreira et P. López-García	<i>C. massiliense</i> (Gourret) Jørgensen	<i>C. ostenfeldii</i> Kofoid, <i>C. protuberans</i> (Karsten) Paulsen	Gourret (1883), 27, T. 1, fig. 2
<i>N. minutum</i> (Jørgensen) F. Gómez, D. Moreira et P. López-García	<i>C. minutum</i> Jørgensen	<i>C. eugrammum</i> Kofoid	Jørgensen (1920), 34, fig. 21-23
<i>N. obesum</i> (Pavillard) F. Gómez, D. Moreira et P. López-García	<i>C. obesum</i> Pavillard		Pavillard (1930), 10, fig. 11
<i>N. pacificum</i> Wood	<i>C. pacificum</i> Wood	non <i>C. pacificum</i> Schröder	Wood (1963), 42, fig. 151
<i>N. paradoxides</i> (Cleve) F. Gómez, D. Moreira et P. López-García	<i>C. paradoxides</i> Cleve		Cleve (1900), 15, T. 7, fig. 14
<i>N. pavillardii</i> (Jørgensen) F. Gómez, D.	<i>C. pavillardii</i>		Jørgensen (1911), 74,

Moreira et P. López-García	Jørgensen		fig. 157, 158
<i>N. pentagonum</i> (Gourret) F. Gómez et P. López-García	<i>C. pentagonum</i> Gourret	<i>C. subrobustum</i> (Jørgensen) Steemann Nielsen	Gourret (1883), 45, T. 4, fig. 58
<i>N. petersii</i> (Steemann Nielsen) F. Gómez, D. Moreira et P. López-García	<i>C. petersii</i> Steemann Nielsen		Steemann Nielsen (1934), 20, fig. 44
<i>N. platycorne</i> (Daday) F. Gómez, D. Moreira et P. López-García	<i>C. platycorne</i> Daday	<i>C. lamellicorne</i> Kofoid	Daday (1888), 101, T. 3, fig. 1, 2
<i>N. porrectum</i> (Karsten) F. Gómez, D. Moreira et P. López-García	<i>C. porrectum</i> Karsten		Karsten (1907), T. 51, fig. 6a,b
<i>N. praeolongum</i> (Lemmermann) F. Gómez, D. Moreira et P. López-García	<i>C. praeolongum</i> (Lemmermann) Kofoid ex Jørgensen	<i>C. gravidum</i> var. <i>praeolongum</i> Lemmermann	Lemmermann (1900), 349, T. 1, fig. 16
<i>N. pulchellum</i> (Schröder) F. Gómez, D. Moreira et P. López-García	<i>C. pulchellum</i> Schröder		Schröder (1906), 358, fig. 27
<i>N. ranipes</i> (Cleve) F. Gómez, D. Moreira et P. López-García	<i>C. ranipes</i> Cleve	<i>C. palmatum</i> (Schröder) Schröder	Cleve (1900), 15, T. 7, fig. 1
<i>N. recurvatum</i> (Schröder) F. Gómez, D. Moreira et P. López-García	<i>C. recurvatum</i> Schröder		Schröder (1906), 367, fig. 40
<i>N. reflexum</i> (Cleve) F. Gómez, D. Moreira et P. López-García	<i>C. reflexum</i> Cleve		Cleve (1900), 15, T. 7, fig. 8, 9
<i>N. schrankii</i> (Kofoid) F. Gómez, D. Moreira et P. López-García	<i>C. schrankii</i> Kofoid		Kofoid (1907b), 306, fig. 29a-31
<i>N. schroeteri</i> (B. Schröder) F. Gómez, D. Moreira et P. López-García	<i>C. schroeteri</i> Schröder		Schröder (1906), 368, fig. 43
<i>N. setaceum</i> (Jørgensen) F. Gómez, D. Moreira et P. López-García	<i>C. setaceum</i> Jørgensen		Jørgensen (1911), 23, fig. 40, 41
<i>N. symmetricum</i> (Pavillard) F. Gómez, D. Moreira et P. López-García	<i>C. symmetricum</i> Pavillard	<i>C. gracile</i> (Gourret) Jørgensen	Pavillard (1905), 52, T. 1, fig. 4
<i>N. tasmaniae</i> (Wood) F. Gómez, D. Moreira et P. López-García	<i>C. tasmaniae</i> Wood		Wood (1963), 42, fig. 152

<i>N. tenue</i> (Ostenfeld <i>et</i> Schmidt) Jørgensen	<i>C. tenue</i> (Ostenfeld <i>et</i> Schmidt) Jørgensen		Jørgensen (1911), 77, fig. 163
<i>N. teres</i> (Kofoid) F. Gómez, D. Moreira <i>et</i> P. López-García	<i>C. teres</i> Kofoid		Kofoid (1907b), 308, T. 29, fig. 34-36
<i>N. trichoceros</i> (Ehrenberg) F. Gómez, D. Moreira <i>et</i> P. López-García	<i>C. trichoceros</i> (Ehrenberg) Kofoid	<i>C. flagelliferum</i> Cleve, <i>C.</i> <i>ramakrishnii</i> Subrahmanyam	Ehrenberg (1859), 791, (1873), 3, T. 1, fig. 1
<i>N. tripos</i> (O.F. Müller) F. Gómez, D. Moreira <i>et</i> P. López-García	<i>C. tripos</i> (O.F. Müller) Nitzsch	<i>C. schroederi</i> Nie, <i>C.</i> <i>neglectum</i> Ostenfeld, <i>C.</i> <i>tripodioides</i> (Jørgensen) Steemann Nielsen	O.F. Müller (1786), 255, T. 22, fig. 18, 1, 3
<i>N. uncinus</i> (Sournia) F. Gómez, D. Moreira <i>et</i> P. López-García	<i>C. uncinus</i> Sournia		Sournia (1972), 74, fig. 6-7
<i>N. vultur</i> (Cleve) F. Gómez, D. Moreira <i>et</i> P. López-García	<i>C. vultur</i> Cleve	<i>C. robustum</i> Ostenfeld <i>et</i> Schmidt	Cleve (1900), 15, T. 7, fig. 5

Figure legends

Figure 1. Light micrographs of *Neoceratium*, subgenus *Tripoceratium*. Specimens collected for the single-cell PCR analysis; see Table 1 for collection date and GenBank accession numbers. **A.** *Neoceratium arietinum*. **B.** *N. concilians*. **C.** *N. contrarium*. **D.** *N. declinatum*. **E.** *N. euarquatium*. **F.** *N. massiliense*. **G.** *N. horridum*. **H-I.** *N. hexacanthum*. **J.** *N. symmetricum*. **K.** *N. platycorne*. **L.** *N. azoricum*. **M.** *N. petersii*. **N.** *N. limulus*. **O.** *N. paradoxoides*. Scale bar = 50 μ m.

Figure 2. Light micrographs of *Neoceratium*, subgenera *Biceratium*, *Amphiceratium* and *Archaeceratium*. Specimens collected for the single-cell PCR analysis; see Table 1 for collection date and GenBank accession numbers. **A.** *N. candelabrum*. **B.** *N. furca*. **C.** *N. kofoidii*. **D.** *N. minutum*. **E.** *N. pentagonum*. **F.** *N. fusus*. **G-H.** *N. extensum*. **I.** *N. gravidum*. **J-L.** *N. digitatum*. Scale bar = 50 μ m.

Figure 3. Light micrographs of the thecal plate pattern of *Ceratium hirundinella* (**A-D**) and *Neoceratium*: *N. furca* (**E-G**), *N. fusus* (**H-I**), *N. tripos* (**J**) and *N. macroceros* (**K-L**). Plates after treatment with sodium hypochlorite, except Fig. 3J. **A-C.** Specimen of *C. hirundinella* at three different focus levels. **B.** Note the plates 6'', 6c and 6'''. **C-D.** Note the three cingular plates in the dorsal side of the cell. **E.** Ventral view showing the first and fourth cingular plates adjacent to the concave ventral area in *N. furca*. **F-G.** Note the two cingular plates in dorsal view in *N. furca*. **H.** Note the two cingular plates in dorsal view and the 4th cingular plate behind the precingular plates in *N. fusus*. **I.** Note the apical and precingular plates. **J.** Detail of the concave ventral area of a live specimen of *N. tripos*. Note the two long sulcal plates bordering the canal of the longitudinal flagellum. **K-L.** Specimen of *N. macroceros* at two different focus levels. **K.** Note the hyaline 6th precingular plate and laterally one or several sulcal platelets. Kofoidian system of tabulation: ' : apical; '' : precingular; c: cingular; s: sulcal; ''': postcingular; ''': antapical; LFI: longitudinal flagellum insertion.

Figure 4. Bayesian inference phylogenetic tree of thecate dinoflagellate SSU rDNA sequences, based on 1096 aligned positions. Species names in bold represent sequences obtained in this study. Numbers at the nodes are posterior probabilities and bootstrap

proportions (values below 0.70 and 75%, respectively, are omitted). Several branches leading to fast-evolving species have been shortened to one third (indicated by 1/3). Accession numbers are provided between brackets. The scale bar represents the number of substitutions for a unit branch length.

Figure 5. Bayesian inference phylogenetic tree of ceratiacean dinoflagellate SSU rDNA sequences, based on 1078 aligned positions. Species names in bold represent sequences obtained in this study. Numbers at the nodes are posterior probabilities and bootstrap proportions (values below 0.70 and 75%, respectively, are omitted). Several branches leading to fast-evolving species have been shortened to one third (indicated by 1/3). Accession numbers of sequences downloaded from GenBank are provided between brackets. The scale bar represents the number of substitutions for a unit branch length.

Figure 6. Resting cysts of *Neoceratium*. **A-B.** *N. candelabrum* from Endoume, Marseille (2 June 2008). **C-E.** *N. furca*, collected from Berre Lagoon (28 July 2008). **D-E.** Different views of other cyst. Scale bar = 50 μm .

Figure 7. Line drawings of the ventral and dorsal views of *Ceratium* (**A-D**) and *Neoceratium* (**E-O**). **A-B.** *Ceratium hirundinella* adapted from Bourrelly (1968, p. 7). **C-D.** *C. cornutum* adapted from Bourrelly (1968, p. 12). **E-F.** *Neoceratium tripos* adapted from Schiller (1937, p. 352). **G-H.** *N. horridum* adapted and modified from Kofoid (1907, p. 180). **I-J.** *N. furca* adapted and modified from Kofoid (1907, p. 179). **K-L.** *N. fusus* adapted and modified from Kofoid (1907, p. 179). **M-O.** *N. pavillardii* adapted from Graham (1942, p. 127). **N.** Detail of the plates of the concave ventral area. Note three (two sutures) and two cingular plates (one suture) in the dorsal views of *Ceratium* and *Neoceratium*, respectively. The three large plates of the concave ventral area are considered to be part of the precingular, cingular and postcingular row of plates as proposed by Wall and Evitt (1975). Kofoidian system of tabulation: ‘: apical; ‘’: precingular; c: cingular; ‘‘’: postcingular; ‘’’’: antapical.













