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ALEXANDRIUM TAMUTUM SP. NOV. (DINOPHYCEAE): A NEW NONTOXIC SPECIES IN THE GENUS *ALEXANDRIUM*[1]

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A new species of the dinoflagellate genus *Alexandrium*, *A. tamutum* sp. nov., is described based on the results of morphological and phylogenetic studies carried out on strains isolated from two sites in the Mediterranean Sea: the Gulf of Trieste (northern Adriatic Sea) and the Gulf of Naples (central Tyrrhenian Sea). Vegetative cells were examined in LM and SEM, and resting cysts were obtained by crossing strains of opposite mating type. *Alexandrium tamutum* is a small-sized species, resembling *A. minutum* in its small size, the rounded-elliptical shape and the morphology of its cyst. The main diagnostic character of the new species is a relatively wide and large sixth precingular plate (6″), whereas that of *A. minutum* is much narrower and smaller. Contrary to *A. minutum*, *A. tamutum* strains did not produce paralytic shellfish poisoning toxins. Phylogenies inferred from the nuclear small subunit rDNA and the D1/D2 domains of the large subunit nuclear rDNA of five strains of *A. tamutum* and numerous strains of other *Alexandrium* species showed that *A. tamutum* strains clustered in a well-supported clade, distinct from *A. minutum*.

Key index words: ***Alexandrium*; *Alexandrium minutum*; *Alexandrium tamarense*; *Alexandrium tamutum* sp. nov.; LSU rDNA; Mediterranean Sea; phylogeny; SSU rDNA, taxonomy**

Abbreviations: **GTX, gonyatoxins; HAB, harmful algal bloom; L:D, light:dark; LSU, large subunit; ML, maximum likelihood; MP, maximum parsimony; NJ, neighbor joining; PSP, paralytic shellfish poisoning; psu, practical salinity units**

An apparent increase in the frequency and intensity of harmful algal blooms (HABs) has fostered research aimed at understanding the taxonomy, ecology, and distribution of the microalgae involved. HAB-forming species often produce potent toxins that can cause serious to fatal illnesses to humans and other vertebrates (Ochoa et al. 1997, Codd et al. 1999, Scholin et al. 2000) and create serious financial losses to local aquacultural activities (Hoagland et al. 2002). Therefore, monitoring programs have been established in many coastal regions, especially those with important mariculture and fishing industries. Proper insight in the taxonomic diversity of HAB-forming species and in their characteristics constitutes a crucial prerequisite to the success of these monitoring programs. Recent studies have revealed that genera with HAB-forming algae are generally far more diverse and more widely distributed than originally believed.

The microalgal genus *Alexandrium* (Dinophyceae, Gonyaulacales) includes 28 described species (Balech 1995, MacKenzie and Todd 2002), eight of which are known to produce paralytic shellfish poisoning (PSP) toxins (Moestrup et al. 2002). PSP events were not an issue for the Mediterranean Sea until about 10 years ago, when the first toxic outbreak attributed to *Alexandrium minutum* was reported for the Spanish Mediterranean coast (Delgado et al. 1990). *Alexandrium minutum*, the type species of the genus, was described from the harbor of Alexandria (Egypt), where it caused intense blooms (Halim 1960). This species has been repeatedly recorded during the last decade in Mediterranean waters, and blooms have been related with PSP toxin production along the Spanish and French coasts and in the Adriatic Sea (Vila et al. 2001a). Two other toxic *Alexandrium* species known from the Mediterranean Sea are *A. andersoni* and *A. catenella*. The first species has been recorded as encysted stage in the Gulf of Naples (Montresor et al. 1998), and PSP

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toxins were found in cultures obtained from cyst germination (Ciminiello et al. 2000), but there have been no reports of PSP events attributable to *A. andersoni* yet. In Europe, chain-forming *A. catenella* was first recorded offshore the coast of Spain in 1983 (Margalef and Estrada 1987), and a number of PSP events along the Spanish and French coasts have been associated with this species in the last years (Vila et al. 2001a,b, Lilly et al. 2002). *Alexandrium tamarense* is responsible for PSP events worldwide and has been recorded along the Spanish, French, and Italian coasts, but there is no evidence of toxic events related to this species along the Mediterranean coasts (Vila et al. 2001a).

Identification and quantification of *Alexandrium* species are difficult when cells are observed in LM. Several species share a similar cell outline and size range. They can be distinguished only by small morphological details of their thecal plates (Balech 1995) made visible in LM upon staining plates with fluorochromes (Fritz and Triemer 1985), after dissecting them under the microscope, or by examination with SEM. Molecular approaches have been used to unravel phylogenetic relationships among the different species of the genus *Alexandrium* (Scholin et al. 1993, Adachi et al. 1994), to trace dispersal patterns of toxic *Alexandrium* species (Anderson et al. 1994, Scholin et al. 1995, Medlin et al. 1998, Lilly et al. 2002), and to infer the biogeographic history of the genus (John et al. 2003b). The detection of species- or strain-specific molecular markers has been widely explored in the last years to gain alternative and precise tools that facilitate the identification of this problematic group of species (Scholin et al. 1994, Adachi et al. 1996a,b, Scholin and Anderson 1996, Anderson et al. 1999, Penna and Magnani 2000, John et al. 2003a).

In the frame of long-term phytoplankton monitoring projects carried out in the Gulf of Trieste (northern Adriatic Sea, Mediterranean Sea) and in the Gulf of Naples (central Tyrrhenian Sea, Mediterranean Sea), a new species of the genus *Alexandrium*, *A. tamutum* sp. nov., has been recorded. We present the results of morphological studies based on culture material by LM and SEM and of the molecular phylogenetic analyses carried out on the small subunit (SSU) and the D1 and D2 domains of the large subunit (LSU) of the nuclear rDNA.

MATERIALS AND METHODS

Cultures. The cultures used for morphological and molecular analysis are listed in Table 1. Morphological investigations were carried out on *A. tamutum* strains isolated from net phytoplankton samples collected in surface waters of the Gulf of Naples (Tyrrhenian Sea) on 18 May 2000 (strains SZN28 and SZN29, maintained in the culture collection of Stazione Zoologica 'A. Dohrn') and in the Gulf of Trieste (Adriatic Sea) on 14 January and 12 March 1997 (strains A8/1, A3 T, and AT5). Two strains of *A. minutum* were also studied: SZN30 isolated from the Gulf of Naples on 18 May 2000 and L20/2 isolated from the Gulf of Trieste on 17 March 1998. Cultures were established by the isolation of a single cell from net phytoplankton samples. Cultures were grown in glass tubes filled with 30 mL of K medium (Keller et al. 1987) prepared with oligotrophic seawater adjusted to a salinity of 36 psu by the addition of double distilled water. Tubes were kept at a temperature of 20° C, a photon flux density of 100 µmol photons $\cdot m^{-2} \cdot s^{-1}$, and a 12:12-h light:dark (L:D) photocycle.

For DNA extraction, unialgal strains of *A. minutum* and *A. tamutum* were grown in 1-L Erlenmeyer flasks in IMR/2 growth medium (Eppley et al. 1967), supplemented with 10 nM selenite, or in K medium in the case of *A. affine*. All cultures were maintained at 15° C in a controlled growth chamber with a 14:10-h L:D photocycle at a photon flux density of 150 µmol photons $\cdot m^{-2} \cdot s^{-1}$.

Morphological observations. Exponentially growing cells were fixed with formaldehyde (0.6% final concentration) and examined and photographed with a Zeiss Axiophot microscope (Carl Zeiss, Oberkochen, Germany) equipped with an AxioCam (Carl Zeiss) photocamera. For plate pattern identification, cells were stained with calcofluor white (Fritz and Triemer 1985) and observed with the same microscope equipped with a UV mercury lamp and a Zeiss 487701 filter set.

For SEM examination, exponentially growing cultures were fixed with formaldehyde (final concentration of 1.6%), dehydrated in a graded ethanol series, critical point dried, and sputter coated with gold. The material was examined with a scanning electron microscope (model 505, Philips Electron Optics BV, Eindhoven, The Netherlands). For plate designation, the notation proposed by Balech (1995) was used.

Cyst production. Cyst production in *A. tamutum* was tested by crossing strains SZN28 and SZN29 (Gulf of Naples) and strains AT3, AT5, and A8/1 (Gulf of Trieste). All strains were inoculated individually and in all pair-wise combinations (to a final concentration of 500 cells $\cdot mL^{-1}$) in single wells of tissue culture plates filled with 8 mL of diluted K medium (K/25). Plates were incubated at a temperature of 20° C, a photon flux density of 50 µmol photons $\cdot m^{-2} \cdot s^{-1}$ provided by cool-white fluorescent bulbs, and a 12:12-h L:D photocycle. Plates were periodically inspected for 6 weeks with LM for cyst production.

Toxin analysis. All cultures of *A. tamutum* and the *A. minutum* strain SZN30 from the Gulf of Naples were tested for PSP toxin production. Cultures were grown in 500-mL Erlenmeyer flasks filled with 250 mL of K culture medium at the same temperature and light conditions used for culture maintenance. Cultures in exponential growth phase were centrifuged and the pellet resuspended in 0.05 M acetic acid. HPLC analyses for saxitoxin, neosaxitoxin, and gonyatoxins (GTX) were carried out following the method of Sullivan and Wekell (1984).

DNA extraction, amplification of rDNA genes, and sequencing. DNA extractions were made from 1 L of culture in logarithmic growth phase, which were harvested with filtration through a 3-µm Isopore membrane filter (Millipore, Schwalbach, Germany) and washed once with sterile seawater. Cells were washed from the filters with 3 mL preheated (65° C) 3% hexadecyltrimethylammonium bromide into 15-mL reaction tubes (Sarstedt, Nünbrecht, Germany), and DNA was extracted following the procedure of Doyle and Doyle (1990). Thereafter the DNA was treated with 10 µL RNase A (10 mg $\cdot mL^{-1}$) (Qiagen, Hilden, Germany) for a 30-min incubated at room temperature, followed by a 90-min incubation in a thermoshaker at 37° C with 20 µL of proteinase K (10 mg $\cdot mL^{-1}$), and purified using phenol:-chloroform extraction with alcohol precipitation. DNA concentration was measured spectrophotometrically at 260 nm, and integrity was verified by agarose-gel electrophoresis. SSU PCR amplification was done with approximately 100 ng DNA in a mix of 86.5 µL water/DNA, 10 µL10 × *Taq* DNA

TABLE 1. List of species and strains used in this study, the GenBank accession numbers for their SSU and LSU sequences, their geographic origin, and the collector and/or culture source.

Species	Strain number	SSU GenBank accession number	LSU GenBank accession number	Geographic origin	Collector, culture source
Alexandrium affine (Inoue & Fukuyo) Balech	PA4V		L38630	Ria de Vigo (Spain)	I. Bravo
	CU1		U44935	Gulf of Thailand	
	CCMP112	AJ535375[a]		Ria de Vigo (Spain)	I. Bravo
Alexandrium andersoni Balech	TC02		U44937	Eastham (USA)	D. Anderson
Alexandrium catenella (Whedon & Kofoid) Balech	BAHME217	AJ535392		Tarragona (Spain)	M. Delgado
	TN9		Scholin et al. (1994)	Tanabe Bay (Japan)	Y. Sako
	WKS-8		Scholin et al. (1994)	Tanabe Bay (Japan)	M. Kodama
Alexandrium concavum (Gaarder) Balech	ALexconc		AF032348	Coromandel (New Zealand)	L. MacKenzie
Alexandrium fundyense Balech	GtCA29	U09048		Gulf of Maine (USA)	
	AFNFA3.1		U44926	Newfoundland (Canada)	D. Anderson
	BGt1		Scholin et al. (1994)	Russian River (CA, USA)	D. Anderson
Alexandrium lusitanicum Balech	GtPort[b]		Scholin et al. (1994)	Portugal	E. Sousa e Silva
Alexandrium margalefi Balech	ALexmarg	U27498	AF033531		
Alexandrium minutum Halim	AL1T		AJ535352	Gulf of Trieste (Italy)	A. Beran
	AL3T[c]	AJ535388	AJ535353	Gulf of Trieste (Italy)	A. Beran
	AL8T		AJ535350	Gulf of Trieste (Italy)	A. Beran
	SZN30[c]	AJ535380[a]	AJ535371[a]	Gulf of Naples (Italy)	M. Montresor
	L20/2		AJ535351	Gulf of Trieste (Italy)	A. Beran
	AL1V		L38625		Zardoya et al. (1995)
	Alexmin1	U27499			
Alexandrium ostenfeldii (Paulsen) Balech & Tangen	AOSH1	AJ535384[a]	AJ535358	Nova Scotia (Canada)	A. Cembella
	Alexostf	U27500			
	K-0324	AJ535381		Limfjord (Denmark)	P. J. Hansen, SCCAP[d]
	K-0287	AJ535382	AJ535356	Limfjord (Denmark)	P. J. Hansen, SCCAP[d]
	BAHME136	AJ535383[a]	AJ535357	Timaru (New Zealand)	N. Berkett
Alexandrium tamarense (Lebour) Balech	Aletamar	AF022191			
	PL173 = CCMP115 = Pgt183	X54946	U44930	Plymouth (UK)	CCMP[e]
	PW06		U44927	Port Benny (USA)	S. Hall
	Orkney1		Medlin et al. (1998)[f]	Orkney Islands (Scotland)	M. Elbrächter
	PE1V		Scholin et al. (1994)[g]	Galicia (Spain)	I. Bravo
	WKS-1		Scholin et al. (1994)[g]	Tanabe Bay (Japan)	M. Kodama
	G.Hope1		U44932	Samchonpo (S.Korea)	G. Hallegraeff
	CU13		U44934	Gulf of Thailand (Thailand)	M. Kodama
	ATBB01		U44933	Bell Bay (Tasmania, Australia)	G. Hallegraeff
	SZN01	AJ535387	AJ535368	Gulf of Naples (Italy)	M. Montresor
	SZN19	AJ535386	AJ535370	Gulf of Naples (Italy)	M. Montresor
	SZN21		AJ535374	Gulf of Naples (Italy)	M. Montresor
	31-4	AJ535391		Cork Harbour (Ireland)	W. Higman
Alexandrium tamiyavanichi Balech	Atamiy	AF113935	AF174614	Straits of Malacca (Malaysia)	G. Usup
Alexandrium tamutum sp. nov.	A8/1[c]	AJ535376[a]	AJ535354[a]	Gulf of Trieste (Italy)	A. Beran
	AT3[c]		AJ535367[a]	Gulf of Trieste (Italy)	A. Beran
	AT5[c]	AJ535377[a]	AJ535366[a]	Gulf of Trieste (Italy)	A. Beran
	SZN28[c]	AJ535378[a]	AJ535373[a]	Gulf of Naples (Italy)	M. Montresor
	SZN29[c]	AJ535379[a]	AJ535372[a]	Gulf of Naples (Italy)	M. Montresor

TABLE 1. Continued.

Species	Strain number	SSU GenBank accession number	LSU GenBank accession number	Geographic origin	Collector, culture source
Alexandrium taylori Balech	AY1T	AJ535390	AJ535347	Lagoon of Marano (Italy)	A. Beran
	AY2T	AJ535385	AJ535348	Lagoon of Marano (Italy)	A. Beran
	AY4T	AJ535389	AJ535349	Lagoon of Marano (Italy)	A. Beran
Fragilidium subglobusum (von Stosch) Loeblich III	fragili		AF260387		
Gonyaulax spinifera (Claparède & Lachmann) Diesing	Gony.spin	AF022155	AF260388		SCCAP[d]
Protoceratium reticulatum (Claparède & Lachmann) Bütschli	Protocer		AF260386		SCCAP[d]
Pyrocystis noctiluca Murray ex Haeckel	Pyrocyst	AF022156		California (USA)	
Tetrahymena thermophila	Tetr.the	X56165			

[a]Sequence produced in this study.
[b]Strain designated also as 18A (Franco et al. 1995).
[c]Strain used for morphological analysis.
[d]Scandinavian Culture Centre for Algae and Protozoa.
[e]Provasoli-Guillard National Centre for Culture of Marine Phytoplankton.
[f]Alignment reported in Fig. 3 of Medlin et al. (1998).
[g]Alignment reported in Fig. 4 of Scholin et al. (1994).

polymerase buffer (Perkin Elmer-Applied Biosystems, Weiterstadt, Germany), 1 μL 1F primer (5′-aac ctg gtt gat cct gcc agt-3′; 10 pmol · μL^{-1}), 1 μL 1528R primer (5-tga tcc ttc tgc agg ttc acc tac-3′; 10 pmol$\mu \cdot L^{-1}$), 1 μL 10 mM dNTPs (Amersham Pharmacia Biotech, Freiburg, Germany), and 0.5 · μL *Taq* DNA polymerase (5 U · μL^{-1}, Perkin Elmer-Applied Biosystems). These are the same primers as in Medlin et al. (1988) but without the restriction sites.

The D1/D2 region of the LSU rDNA was amplified using the same PCR reaction mix as described above but using the DIRF primer (5′-acc cgc tga att taa gca ta-3′) and D2CR primer (5′-cct tgg tcc gtg ttt caa ga-3′), according to Scholin et al. (1994). The PCR reaction was carried out in a Mastercycler Gradient (Eppendorf, Hamburg, Germany) with an initial denaturation of 94° C for 4 min followed with 30 cycles of 94° C for 2 min (denaturation), 56° C for 2 min (annealing), and 72° C for 2 min (extension). PCR reactions were carried out in triplicate. The triplicates were pooled, purified with Qiagen PCR purification kit, and then sequenced using the Long Read kit (Biozym, Hessisch Oldendorf, Germany) on a LiCor 4000L automatic sequencer (MWG, Ebersberg, Germany), according to the manufactures instructions. For the SSU rDNA sequencing reaction, we used the following primers, which were optimized for use on a LiCor sequencer with a 5′ IRD 800 modification. These are slightly different (longer/shifted) than the original versions published in Elwood et al. (1985): 1F primer (5′-aac ctg gtt gat cct gcc agt-3′), 528F (5′-gcg gta att cca gct cca a-3′), 1055F (5′-ggt ggt gca tgg ccg ttc tt-3′), 536R (5′-aat tac cgc ggc kgc tgg ca-3′), 1055R (5′-acg gcc atg cac cac cac cca t-3′), 1528R (5′-tga tcc ttc tgc agg ttc acc tac-3′). For D1/D2 region of the LSU, we used the DIRF primer (5′-acc cgc tga att taa gca ta-3′) and 739R (5′-ggt ccg tgt ttc aag acg gg-3′).

Phylogenetic analyses. Sequence alignment was done with Clustal × software (Thompson et al. 1997) and improved by eye for both the SSU sequence and the LSU sequence. Alignments are available at GenBank or have been published in the literature (Table 1). For the SSU rDNA we used a data set containing 28 taxa and 1751 bp with *Tetrahymena thermophila*, *Gonyaulax spinfera*, and *Pyrocystis noctiluca* as outgroup species. An optimal base substitution model was calculated using Modeltest (Posada and Crandall 1998, 2001). Maximum likelihood (ML) analyses were constrained with the optimal parameters as obtained by hierarchical likelihood ratio tests (hLRTs). ML phylogenies were reconstructed with PAUP* 4.0b8 (Swofford 1998) constrained with the following Modeltest parameters. hLRTs gave a general time reversible (GTR) model, allowing for invariant sites and a gamma distribution (GTR + I + G) as the model that fit best the data set. The ML-tree calculation was constrained using base frequencies of A = 0.27665, C = 0.1779, G = 0.2547, T = 0.2908; base substitution frequencies of A-C = 0.9683, A-G = 2.6044, A-T = 1.1677, C-G = 0.6080, C-T = 5.2852, G-T = 1.0000; proportion of invariable sites I = 0.2652; and a gamma distribution shape parameter = 0.6411.

The data set for the D1/D2 region of the LSU rDNA contained 42 taxa and 635 unambiguous nucleotide positions. The tree was rooted using *Fragilidium subglobosum*, *Protoceratium reticulatum*, and *G. spinifera* as multiple outgroups. ML phylogenies were reconstructed with PAUP* 4.0b8 (Swofford 1998). The selected model, using hLRTs for the partial LSU rDNA data set, was the GTR model with a gamma distribution (GTR + G); base frequencies of A = 0.2657, C = 0.1647, G = 0.2482, T = 0.3214; base substitution frequencies of A-C = 0.6874, A-G = 1.7143, A-T = 0.7864, C-G = 0.4543, C-T = 3.3605, G-T = 1.0000; proportion of invariable sites I = 0; and gamma distribution shape parameter = 0.7306.

Nodal support was estimated by bootstrap analyses (Felsenstein 1985) using maximum parsimony (MP), neighbor joining (NJ) with ML distances, and ML analyses with the model

parameters described above. The bootstrap analyses were done in 1000 replicates for MP and NJ and with 100 replicates for the ML analysis.

RESULTS

Alexandrium tamutum Montresor, Beran and John sp. nov.

Fig. 1, A–B, Fig. 2, A–C, Fig. 3, A–G, Fig. 4, A–B

Cellulae longae 19–33.7 μm et latae 19–32.6 μm, forma rotunda usque ad ellipticam. Cingulum excavatum est et 1/1 sua latitudine sub laeva parte descendit. Formula laminarum: Po, 4′, 0a, 6″, 6C, 9S, 5‴, 0p, 2⁗. Prima apicalis lamina, rhombi inaequalis forma, cum lamina Po coniuncta est. Parvum ventrale foramen in anteriore dextero margine primae apicalis laminae adest. Sexta praecingularis lamina (6″) tam lata quam longa est. Sulci posterior lamina (Sp), fere rectianguli forma, magis lata quam longa est. Thecae laminae subtiles et leves sunt cum parvis poris. Rotundae cistes sunt (diameter = 26–32 μm) cum a summo videntur et renis forma (altitudo = 16–20 μm) cum iuxta videntur. Cistis paries spissus est et corio mucoso circumdatus; id quod cistis continet granosum est et stigma, colore quasi fusco, adest.

Cells 19–33.7 μm long and 19–32.6 μm wide, round to elliptical in shape. Cingulum excavated with its right end displaced posteriorly one cingular width. Plate pattern: Po, 4′, 0a, 6″, 6C, 9S, 5‴, 0p, 2⁗. The first apical plate is irregularly rhomboidal and contacts Po. A small ventral pore is present on the anterior right margin of the first apical plate. The sixth precingular plate (6″) is as wide as long. The posterior sulcal plate (Sp) is roughly rectangular, wider than longer. Thecal plates are thin and smooth, with small pores. Cysts are circular (26–32 μm diameter) when seen from above and kidney shaped (16–20 μm high) when seen from the side. Cyst wall is thick and surrounded by a mucous layer; cyst content is granular and a brownish accumulation body is present.

Habitat: Marine

Type locality: Gulf of Naples, Tyrrhenian Sea, Mediterranean Sea

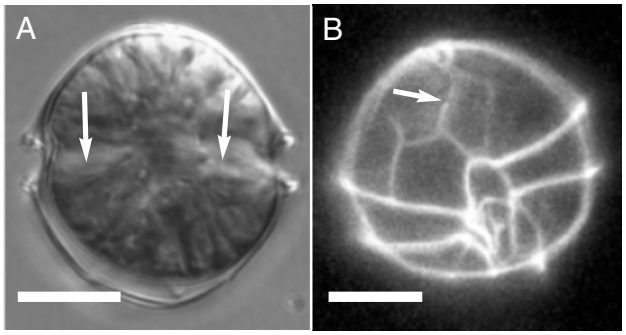

FIG. 1. *Alexandrium tamutum* sp. nov., light micrographs of vegetative cells. (A) Nomarski optics micrograph of a cell in ventral view (strain SZN28) the two ventral arms of the horseshoe-shaped nucleus are shown by an arrow. (B) Calcofluor-stained cell of strain AT5 in ventral view; the ventral pore is shown by an arrow. Scale bars, 10 μm.

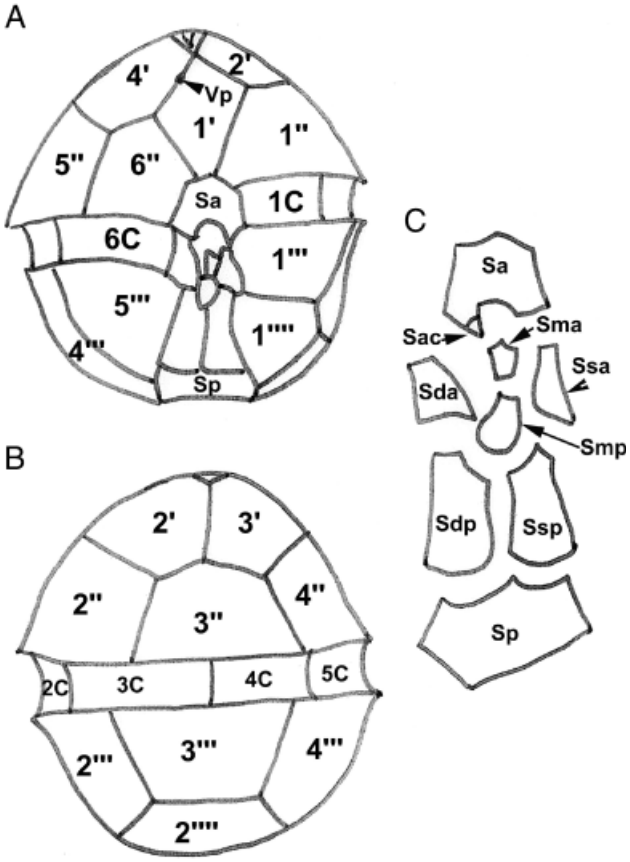

FIG. 2. *Alexandrium tamutum* sp. nov., schematic drawings of thecal plate patterns. (A) Ventral view, (B) dorsal view, (C) sulcal area. For abbreviations, see Figure 3.

Holotype: Figure 3A

Iconotype: Figure 2, A–C

Etymology: The word *tamutum* is composed by the first part of the word "*tamarense*" and the last part of the word "*minutum*" and indicates the morphological relatedness of *A. tamutum* with both *A. tamarense* and *A. minutum*.

Cell shape is rounded to elliptical (Figs. 1, 2, A and B, and 3, A and B), but larger cells in ventral view can have a slightly pentagonal outline (Fig. 3A). Cell size ranges from 19 to 33.7 μm in length and from 19 to 32.6 μm in width (Table 2); cell depth is almost equal to cell width. The epitheca is dome shaped, almost hemispherical in most cells, but it can be helmet shaped in larger cells because of a change in curvature of the epitheca toward the cingulum. The hypotheca is hemispherical to almost trapezoidal when cells are slightly tilted; the antapical margin of the hypotheca in correspondence to the sulcal area is flat and the left portion of the hypotheca is slightly protruding (Fig. 3D). The cingulum is excavated, descending its own width, and is bordered by very narrow cingular lists (Figs. 1B, 2A, and 3, A, B, D, and E). The sulcus is slightly depressed, with narrow sulcal lists, the left list wider than the right one (Fig. 3, A and D). When cells are slightly tilted, the

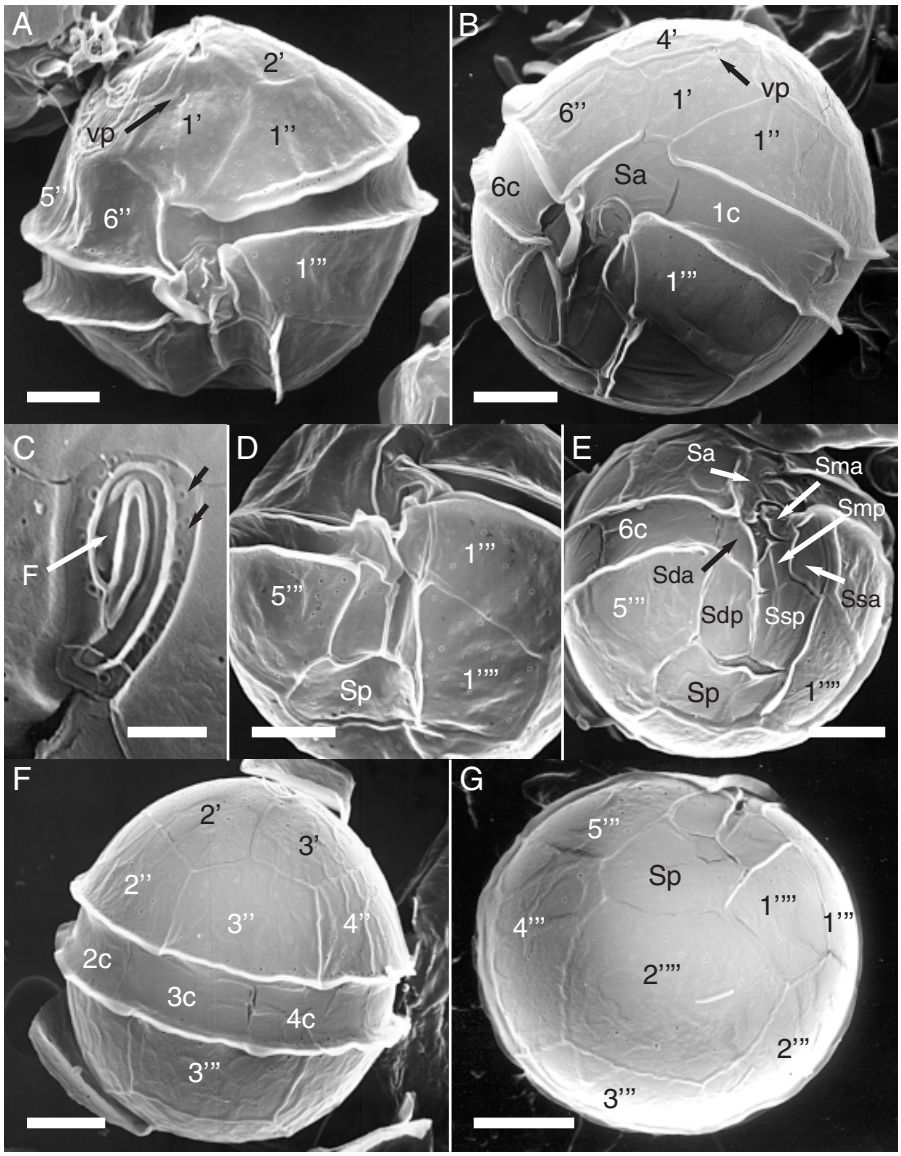

Fig. 3. *Alexandrium tamutum* sp. nov., SEM micrographs of vegetative cells. 1′ up to 4′, apical plates; vp, ventral pore; 1″ up to 6″, precingular plates; 1c, up to 5c, cingular plates: Sa, sulcal anterior plate; Sma, sulcal median anterior plate; Smp, sulcal median posterior plate; Sda, sulcal right anterior plate; Ssa, sulcal left anterior plate; Sdp, sulcal right posterior plate; Ssp, sulcal left posterior plate; Sp, sulcal posterior plate; 1‴ up to 5‴, postcingular plates; 1⁗ and 2⁗, antapical plates. (A) Ventral view (strain SZN28). (B) Ventral view of a slightly expanded cell (strain A8/1). (C) Apical pore (strain A8/1), the foramen (F) is shown by an arrow: black arrows indicate marginal pores. (D) Sulcal area (strain A8/1). (E) Sulcal area of a slightly expanded cell (strain A8/1). (F) Dorsal view (strain A8/1). (G) Antapical view (strain A8/1). Scale bars: A, B, D–G, 5 μm; C, 1 μm.

two very small projections of the sulcal lists are visible at the antapical margin of the hypotheca. Cells appear smooth in LM (Fig. 1). Small scattered pores are visible on the thecal plates when cells are observed at SEM (Fig. 3). Cells have numerous brownish chloroplasts and a horseshoe-shaped nucleus located in the equatorial portion of the cell (Fig. 1A).

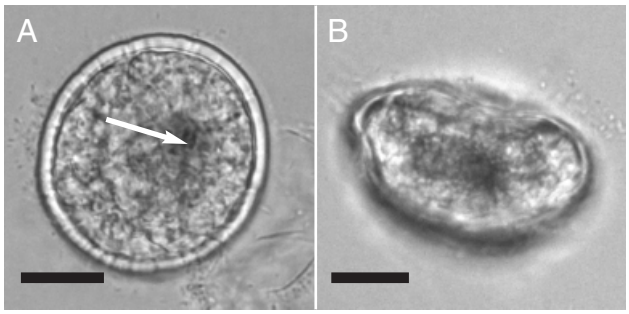

Fig. 4. *Alexandrium tamutum* sp. nov., light micrographs of resting cysts. (A) Round cyst seen from above; the accumulation body is shown by an arrow. (B) Kidney-shaped outline of a cyst in lateral view. Scale bars, 10 μm.

The plate formula is Po, 4′, 0a, 6″, 6C, 9S, 5‴, 2⁗ (Fig. 2). The pore plate (Po) is slightly convex on its left margin and straight on its right margin (Fig. 3C). The canopy is narrow; the foramen (or comma) is bordered by well-developed margins. Marginal pores are present on the pore plate (Fig. 3C). The ventral end of Po is truncated and in contact with plate 1′ (Fig. 3, A-C). Plate 1′ has the typical rhomboidal shape with truncated anterior and posterior angles and two longer (anterior right, posterior left) and two shorter sides (Figs. 1B, 2A, and 3, A and B). The posterior margin of this plate contacts the anterior sulcal plate (Sa). A small ventral pore is located on the anterior half of the anterior right margin of plate 1′ (Figs. 1B, 2A, and 3, A and B). Plate 6″ is wide, with a width:length ratio ≅ 1

TABLE 2. Dimensions of *Alexandrium tamutum* sp. nov. strains.

Strains	Length[a] (μm)	Minimum length	Maximum length	Width[a] (μm)	Minimum width	Maximum width
A8/1	26.0 (3.4)	21.5	31.4	25.0 (2.7)	20.9	30.2
AT5	26.3 (3.3)	22.1	33.1	25.2 (3.1)	19.8	32.6
AT3	26.4 (2.6)	22.1	33.7	25.7 (2.3)	21.5	32.6
SZN28	28.0 (2.7)	19.0	34.0	27.5 (2.7)	19.0	32.0
SZN29	27.3 (2.9)	20.5	32.0	26.6 (3.4)	20.0	32.0

For each strain, 30 exponentially growing cells were measured.
[a]Values in parentheses are SD.

(Figs. 1B, 2B, and 3, A and B). Cingular plates are similar in size and are ornamented by very small poroids along their anterior and posterior margins. The sulcus is composed of nine sulcal plates (Figs. 2C and 3, D and E). The anterior sulcal plate (Sa) is as long as wide, with a straight anterior margin that contacts the posterior side of plate 1′. The left margin of Sa is straight and in contact with plate 1C. The right margin is oblique and in contact with 6″. The left anterior sulcal plate (Ssa) is narrow and almost rectangular. The right anterior sulcal plate (Sda) is trapezoidal in shape and bears a small list on its left side, toward the ventral part of the cell. The anterior sulcal median plate (Sma) is small, almost ellipsoidal, and is located underneath the Sa; the posterior sulcal median plate (Smp) is slightly longer that wide. One very small arrowhead-shaped sulcal accessory platelet (Sac) is located at the posterior left end of the Sa plate and is completely hidden by the list of the Sda. The right and left posterior sulcal plates (Sdp and Ssp) are similar in shape and longer than wide. The posterior sulcal plate (Sp) is roughly rectangular, wider than longer (Figs. 2C and 3, E and G). The anterior margin of plate Sp is subdivided into two parts, which represent the borders with the left and right posterior sulcal plates. In the hypotheca, plate 1‴ bears a thin list along its right side in contact with the sulcal area (Fig. 3, A, B, D, and E). Plate 2‴ is pentagonal and large (Fig. 3G).

Resting cysts were obtained only by crossing cultures SZN29 and SZN28, both isolated from the Gulf of Naples. Cysts of *A. tamutum* have an average diameter of 29.8 μm ($n = 30$; minimum diameter, 26 μm; maximum diameter, 32 μm). They are almost hemispherical in shape, with a circular outline when observed from above (Fig. 4A) and kidney shaped (Fig. 4B) in lateral view (16–20 μm high). Cysts contain granular material and an orange accumulation body (Fig. 4A). The cyst wall is thick and surrounded by a layer of mucous material by which they stick to the bottom of the culture plate.

In the Gulf of Naples, *A. tamutum* and *A. minutum* strains were isolated from the long-term sampling station MareChiara located 1 mile offshore. The strains used in the present investigation were isolated in May, when the seasonal thermocline was located at 15 m, surface water temperature was around 24° C, and salinity was 37.5 psu. Both species were found in net samples, whereas they were absent from the seawater samples settled for quantitative analyses. The *A. minutum* strain presented the typical features described for the species. Cells were small (18–25 μm in length), with a smooth thecal plate, a very narrow 6′ (precingular plate), and a rhomboidal 1' with the ventral pore located on the posterior side of the right anterior margin (Fig. 5). In the Gulf of Trieste, the two species were isolated in January and March, with temperatures ranging between 9.9 and 10.6° C and salinity between 37.2 and 37.5 psu.

Toxin analyses. All strains of *A. tamutum* tested for PSP toxins with HPLC analyses showed no toxin content. Strain SZN30 (*A. minutum*) showed the presence of the following PSP toxins: neosaxitoxin ($2.45\,fg \cdot cell^{-1}$), GTX1 ($193\,fg \cdot cell^{-1}$), GTX2 ($10.7\,fg \cdot cell^{-1}$), GTX3 ($4.73\,fg \cdot cell^{-1}$), and GTX4 ($171\,fg \cdot cell^{-1}$).

Phylogenetic analysis. The SSU rDNA phylogeny shows that the genus *Alexandrium* is monophyletic (Fig. 6). Within *Alexandrium*, *A. taylori* diverged first, followed by *A. margalefi*. Thereafter, the remaining taxa diverged in two major clades. The first clade, supported with moderate to strong bootstrap values, includes *A. minutum*, *A. ostenfeldii*, and *A. tamutum*. The new species *A. tamutum* is a sister taxon to *A. ostenfeldii*, and both are sisters to *A. minutum* strains. The second major cluster contains *A. affine*, followed by the later divergence of *A. tamiyavanichi* and then

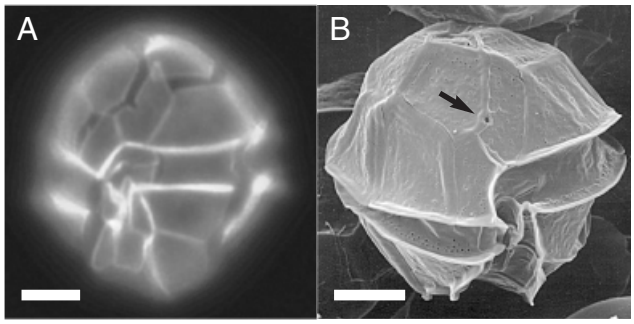

FIG. 5. *Alexandrium minutum*, vegetative cells of strain SZN30. (A) Light micrograph of a vegetative cell. (B) SEM micrograph of a vegetative cell in ventral/apical view; the ventral pore is shown by an arrow. Scale bars, 5 μm

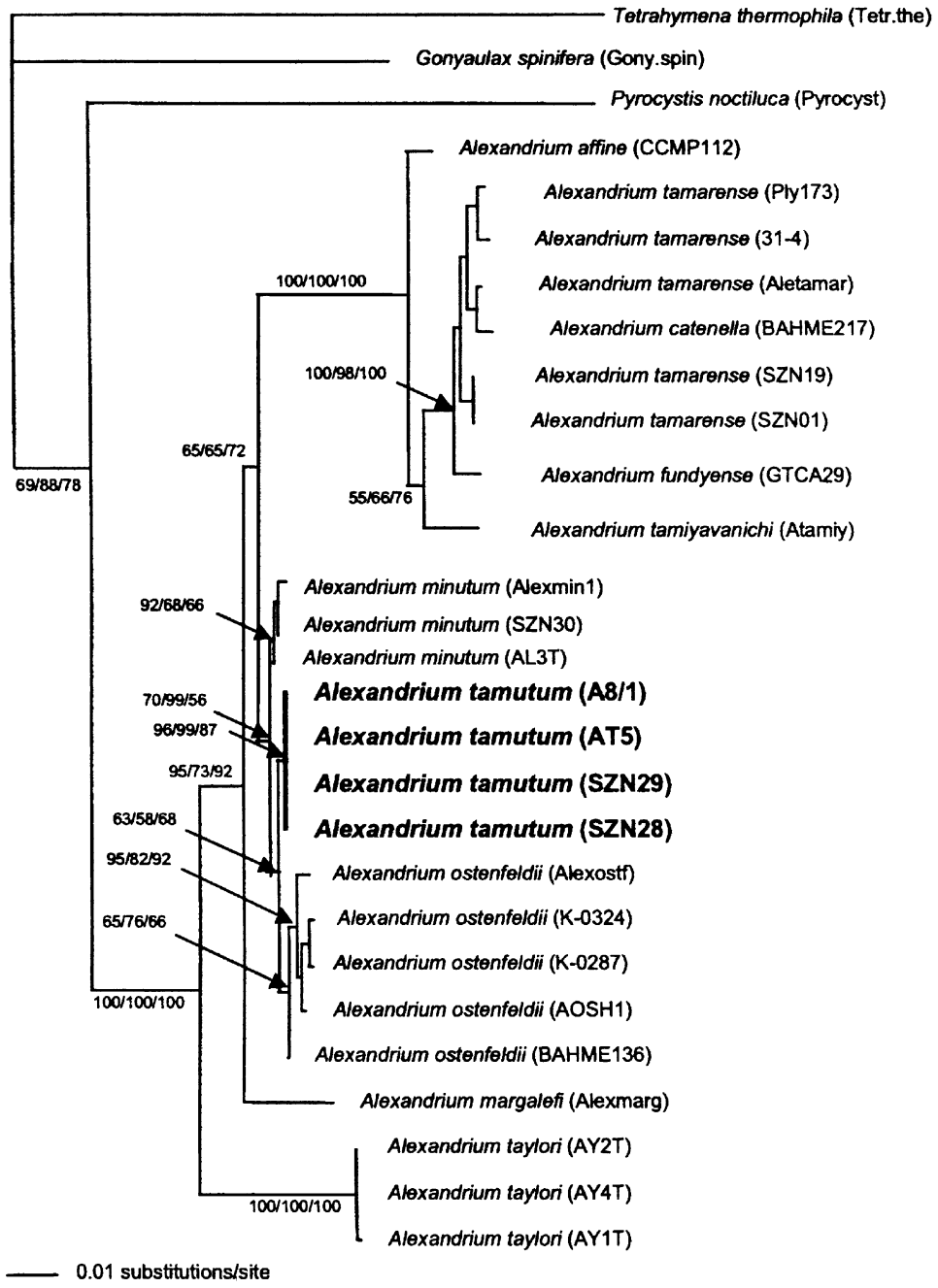

FIG. 6. ML phylogenetic tree of SSU rDNA sequences of the genus *Alexandrium*. *Tetrahymena thermophila*, *Gonyaulax spinifera*, and *Pyrocystis noctiluca* were used as outgroups. MP/NJ/ML bootstrap values (>50%) are placed close to each node or to an arrow directing toward a node. The MP tree had a length of 1074 steps, a 0.7933 consistency index, and 0.8323 retention index.

the *A. tamarense* "species complex" *sensu* Scholin et al. (1995).

The phylogenetic tree inferred from the D1/D2 region of the LSU rDNA (Fig. 7) differed from the SSU tree in the following aspects. The ML tree shows three lineages, the first one, comprising *A. taylori* and *A. margalefi*, diverging before two major sister lineages, which have a similar topology to the SSU tree but include more species. The first of the sister lineages shows the previously known topology of a first divergence of *A. affine* and *A. concavum*, followed by *A. tamiyavanichi* and then the *A. tamarense* species complex. The *A. tamarense* species complex reflects the different geographic clades and not the three morphotypes (*A. fundyense*, *A. catenella*, *A. tamarense*) known to occur in this species complex. The second of the sister lineages consists of *A. andersoni*, diverging before the lineage splits again into two clades. One of these two clades includes *A. minutum/lusitanicum* and the other includes *A. tamutum* and *A. ostenfeldii*. Most clades of the SSU tree and most of those in the LSU tree showed moderate to good bootstrap support for the branching order of the younger major lineages. In both trees the shallower clades, corresponding to

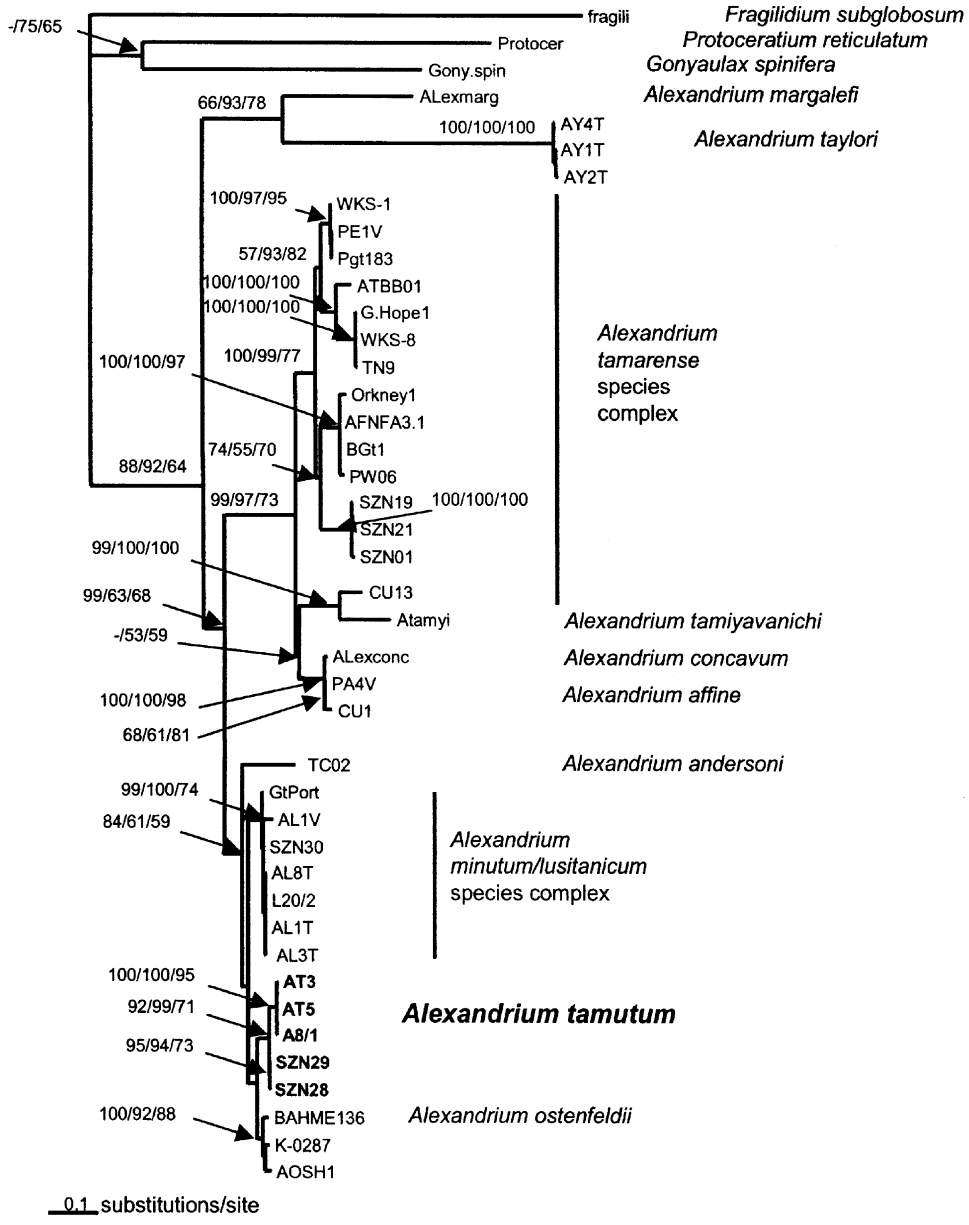

FIG. 7. ML phylogenetic tree of representatives of the genus *Alexandrium* based on their sequences of the D1/D2 region of the LSU rDNA. *Fragilidium subglobosum*, *Protoceratium reticulatum*, and *Gonyaulax spinifera* were used as outgroups. MP/NJ/ML bootstrap values (>50%) are placed close to each node or an arrow directing toward a node. The MP tree had a length of 1382 steps, a 0.6143 consistency index, and 0.8071 retention index

species groups or species complexes (see above), were well supported by bootstrap values. However, the low bootstrap support for the nodes of the *A. minutum/lusitanicum*, *A. tamutum*, and *A. ostenfeldii* clade in the phylogenetic tree of the D1/D2 region did not resolve relationships among the three species.

DISCUSSION

Results of morphological investigations reveal that *A. tamutum* is a new species. Although it shares several morphological characters with *A. minutum* (e.g. small size, shape of the sulcal posterior plate, cyst morphology), it is distinguishable from the latter species by the larger size and width of its sixth precingular plate (6″). Moreover, results of HPLC analyses for PSP toxin detection showed that *A. tamutum* strains do not produce PSP toxins, whereas *A. minutum* generally does (Hallegraeff et al. 1988, Oshima et al. 1989, Franco et al. 1994, Chang et al. 1997, this investigation). Results of phylogenetic inferences on the SSU and partial LSU of the nuclear rDNA support the recognition of *A. tamutum* as a distinct species. Strains isolated from coastal waters in two Mediterranean sites (Gulf of Naples, Tyrrhenian Sea and Gulf of Trieste, Adriatic Sea) clustered together with high bootstrap values and were not intermingled with isolates of *A. minutum* from different geographic areas.

Morphology. Species of the genus *Alexandrium* share a plate pattern of Po, 4′, 0a, 6″, 6c, 9–10 s, 5″,

TABLE 3. Selected morphological characters of *Alexandrium tamutum* sp. nov. and morphologically closely related species of the genus *Alexandrium*.

Character	*A. tamutum*	*A. minutum*[a]	*A. tamarense*[a]	*A. camurascutulum*[b]	*A. andersoni*[a]	*A. angustitabulatum*[a]
Cell shape	Oval to elliptical to Slightly pentagonal	Oval to elliptical	Irregularly pentagonal	Oval	Oval to elliptical	Oval to elliptical
Cell length, µm	19–34	15.5–29 (most: 21–26)	22–51	26–28	21–35	17.5–24
Cell width, µm	19–30.2	15.5–29 (most: 21–26)	22–50	21–24	18–33	17.5–24
Apical attachment Pore on Po	Absent	Absent	Absent/at times present (small)	Present (large)	Absent	Absent
1′ shape	Anterior right margin straight	Narrow with concave anterior right margin	Concave anterior right margin		Narrow	Posterior right and anterior left margins very short
Contact between Po and 1′	Present	Present/absent	Present	Absent	Present/absent	Present
Location of the ventral pore on the anterior right side of 1′	Middle to anterior part	Posterior part	Variable	Lower third	Middle	Absent
6″ plate	Wide (w ≅ l)	Narrow (w<l)	Wide (w ≅ l)	Wide (w>l) Hooked shape	Wide in its middle side	Narrow (w<l)
Sa plate	w ≅ l	w ≅ l	w ≅ l	w ≅ l	Trapezoid	w ≅ l
Sp plate	w>l	w>l	w<l	w ≅ l	w>l	w>l
Posterior attachment pore on Sp	Absent	Absent	Absent/present	Present	Absent	Absent
Plate ornamentation	Small pores	Small pores; at times reticulated hypotheca	Small pores	Pores with areolae	Pores with areolae	?
Cyst shape	Hemispherical	Hemispherical[c]	Elliptical[d]	Unknown	Round[e]	Unknown

w, width; l, length.
[a]Balech (1995).
[b]MacKenzie and Todd (2002).
[c]Bolch et al. (1991).
[d]Dale (1977).
[e]Montresor et al. (1998).

0p, 2‴′ (Balech 1995). Species identification within this genus is mainly based on morphological characters, such as cell shape, size, chain formation, presence or absence of a ventral pore, and shape and size of some specific thecal plates, namely, first apical, pore plate, sixth precingular, anterior and posterior sulcal plates. Most of these characters are visible only upon dissecting thecal plates under the microscope, staining them with fluorochromes (Fritz and Triemer 1985), or after the examination of cells at SEM. The painstaking study in LM of hundreds of samples from all over the world allowed Balech (1995) to set a comprehensive classification framework for *Alexandrium* species, based on the combination of several morphological characters of the vegetative cells. The genus presently includes 28 species plus a number of species/morphotypes, which still need to be investigated to assess definitively their taxonomic status (Balech 1995, MacKenzie and Todd 2002).

Strains of *A. tamutum* present morphological characters, which are shared at times by *A. minutum* and at times by *A. tamarense* (Table 3). The cell outline of *A. tamutum* is generally rounded or elliptical. This, together with its relatively small size, at first sight is reminiscent of *A. minutum*, to one of the small-sized species included in the "*A. minutum* group," or to the recently described *A. camurascutulum* (Table 3) (Balech 1995, MacKenzie and Todd 2002). However, *A. tamutum* cells in their larger size range present a slightly pentagonal shape, thus reflecting the outline of small-sized *A. tamarense*. The above-mentioned species have distinct size ranges, but they overlap widely; thus, cell size is not a useful character for distinguishing *Alexandrium* species.

The large size of plate 6″ in the precingular series, with a width:height ratio ≈ 1, differs from that described for *A. minutum* and is most similar to the 6″ plate described for *A. tamarense* (Table 3). This is the main morphological character that distinguishes our new species from *A. minutum*. A distinctive morphological feature of this latter species is in fact the very narrow shape and reduced size of the sixth precingular plate (Balech 1989, 1995). The sulcal posterior plate (Sp) of *A. tamutum* is of the "*minutum* type," viz. roughly rectangular in shape and wider than high, whereas the Sp of *A. tamarense* is distinctively higher than wide (Balech 1995). The small-sized *A. camurascutulum* also presents a wide 6" plate, which, however, is wider than long and has a characteristic hooked shape because of the strongly curved left anterior margin (MacKenzie and Todd 2002). Moreover, this latter species can be distinguished from *A. tamutum* and the other species included in the *minutum* group by a longer sulcal posterior (Sp) plate and by the presence of a large attachment pore on both the sulcal posterior plate and the pore plate (Table 3).

The pore plate of *A. tamutum* resembles that of *A. minutum*, with an almost undetectable callus and small marginal pores surrounding the foramen (or comma). Plate 1′ has a small ventral pore on its anterior right side. However, whereas in *A. minutum* the ventral pore is located on the posterior end of the anterior right margin of plate 1′ (Balech 1995), in *A. tamutum* the pore is situated in the median/upper part of the margin. In all the strains of *A. tamutum* examined, the connection between the pore plate and plate 1′ is clearly visible, whereas in *A. minutum* this connection is at times hidden by the overgrowth of the margins of plates 2′ and 4′.

The hypothecal plates of *A. tamutum* are smooth, whereas those of *A. minutum* in the type locality (Balech 1989) and the two strains of *A. minutum* we examined here also had a smooth hypotheca. Specimens attributed to *A. minutum* with evident hypothecal ornamentation have been reported for natural material collected in the Gulf of Naples and Japanese waters (Montresor et al. 1990, Yuki 1994, Balech 1995). It is not known if the microreticulation is a stable character or if it can be lost in culture conditions, and no molecular data relative to a reticulated morphotype of *A. minutum* are available to our knowledge. Montresor et al. (1990), reporting on *Alexandrium* species from the Gulf of Naples, illustrated a cell of *A. minutum* characterized by a narrow 6" plate and a strongly reticulated hypotheca (their fig. 3b). However, they also illustrated a cell (their fig. 3a) that instead had a wide sixth precingular plate and should probably be attributed to *A. tamutum*.

The cyst. The resting cysts of *A. tamutum* are morphologically identical to those of *A. minutum* (Bolch et al. 1991). Almost all *Alexandrium* cysts known up to now are ellipsoidal (*A. tamarense*, *A. catenella*, *A. cohorticula*), spherical (*A. affine*, *A. leei*, *A. monilatum*, *A. ostenfeldii*, *A. taylori*), or approximately hemispherical (*A. minutum*, *A. lusitanicum*, *A. hiranoi*) in shape, with a smooth wall surrounded by a more or less developed mucous layer and deprived of any morphological feature that could help for their identification at the species level (Bolch et al. 1991, MacKenzie et al. 1996, Garcés et al. 1998). The only exception to the smooth-walled cysts is represented by the cyst of *A. pseudogonyaulax*, which presents clear markings reflecting the tabulation of the vegetative cell (Montresor et al. 1993, Montresor 1995).

The presence of a heterothallic life cycle has been reported for *A. catenella* (Yoshimatsu 1981), whereas for *A. tamarense* a more complex probably multiple mating type system has been suggested (Destombe and Cembella 1990). In *A. tamutum*, resting cysts were never observed within cultures established from the isolation of a single cell but only after crossing strains of different mating type. Interestingly, cyst formation was only obtained by crossing strains from the Gulf of Naples, whereas no cysts were observed in crossings among the isolates from the Gulf of Trieste or among them and the Neapolitan strains. Cyst formation can be interpreted as evidence for sexual reproduction and thus mating compatibility between two strains, but the lack of cyst formation cannot be considered as evidence for lack of

sexual reproduction. Several possible factors could explain the lack for cyst formation. The strains of *A. tamutum* isolated from the Adriatic Sea could have belonged to the same mating type, and thus the crossing among them should have been ineffective. However, if *A. tamutum* has a simple heterothallic life cycle with mating types of two opposite signs, the Adriatic strains should have been compatible with one of the two Neapolitan strains. An alternative hypothesis is the presence of multiple mating types in *A. tamutum* as in *A. tamarense* and *Gymnodinium catenatum* Graham (Destombe and Cembella 1990, Blackburn et al. 2001). We can also hypothesize that mating barriers start to establish between strains from the Gulf of Trieste and those from the Gulf of Naples as suggested by the separation of the two strains in the phylogenetic tree based on LSU sequences. Ultimately, sexual reproduction (i.e. conjugation and zygote formation) could have occurred but without leading to encystment, perhaps because the required conditions were not met in the experimental condition we used.

Toxicity. HPLC analyses showed that *A. tamutum* strains from both the Gulfs of Naples and Trieste do not produce PSP toxins, whereas the two strains of *A. minutum* are toxic (Tillmann and John 2002, this investigation). The first HPLC analyses carried out on *A. minutum* detected the presence of GTX1, 2, 3, and 4 (Oshima et al. 1989). However, notable variability in toxin content and relative proportions has been observed in strains from different geographic areas or in different culture conditions (Franco et al. 1994, Béchemin et al. 1999, Hwang and Lu 2000), and isolates from New Zealand showed neosaxitoxin as the principal component (Chang et al. 1997). Small amounts of this toxin were also detected in the Neapolitan strain of *A. minutum*, whereas it was not detected in the strain isolated from the Gulf of Trieste (Tillmann and John 2002). It is interesting to note that another strain of *A. minutum* (AL1 T), isolated from the Gulf of Trieste and included in the present LSU phylogeny, was not found to produce PSP toxins (Tillmann and John 2002). This is, to our knowledge, the first report of a non–PSP-producing *A. minutum*.

Phylogenetic relationships within the genus Alexandrium. Phylogenetic analyses carried out on the SSU and LSU of the nuclear rDNA of multiple strains of different *Alexandrium* species showed that the addition of strains of the new species *A. tamutum* does not change the basic topology of trees obtained in previous investigations (John et al. 2003b). *Alexandrium taylori* and *A. margalefi* are at the base of the *Alexandrium* clade, which then splits into two major clades: one grouping species of the *A. tamarense* species complex, *A. tamiyavanichi* and *A. affine*, and the other including *A. minutum*, *A. tamutum*, and *A. ostenfeldii*. Molecular phylogenies failed to support supraspecific groupings suggested for the genus *Alexandrium* (Balech 1995) and do not help us in understanding which of the morphological characters we currently use for morphological investigations have to be considered ancestral or derived.

In the taxonomic revision of the genus *Alexandrium*, Balech (1995) grouped some of the species sharing peculiar morphological features within supraspecific "groups." One of them is the *minutum* group (*A. andersoni*, *A. angustitabulatum*, *A. minutum*, and *A. lusitanicum*) that includes species generally less than 30 μm in size, with a short, wider than long, posterior sulcal plate (Sp) and a very narrow sixth precingular plate (6″). Our molecular analyses, which include all but one (*A. angustitabulatum*) species of the *minutum* group, showed no phylogenetic support for this supraspecific clustering. Strains of *A. minutum* and *A. lusitanicum* group together in a well-supported clade, confirming that they should be considered the same species, as already hypothesized by Balech and suggested by other authors (Balech 1995, Costas et al. 1995, Franco et al. 1995). However, *A. andersoni* does show close phylogenetic relationship with *A. minutum*, branching out at the base of the *A. minutum–A. tamutum–A. ostenfeldii* clade.

Phylogenetic studies within the genus *Alexandrium* have been mainly focused on the so-called *A. tamarense* species complex, which groups three morphospecies, *A. tamarense*, *A. fundyense*, and *A. catenella*, responsible of harmful events worldwide. These three species are distinguished based on the combination of two main characters: presence (*A. tamarense*) or absence (*A. fundyense*, *A. catenella*) of a ventral pore on the first apical plate and formation (*A. catenella*) or lack of formation (*A. tamarense*, *A. fundyense*) of long chains of cells (Balech 1995). However, molecular analyses carried out on the variable domains of the LSU rDNA of a large number of strains from different geographic areas showed that the different morphospecies clustered together based on their biogeographic origin and not on their morphological features (Scholin et al. 1994, 1995, Medlin et al. 1998, Higman et al. 2001, John et al. 2003b). This pattern has been explained with the evolution of genetically distinct populations via geographic isolation coupled by human-mediated dispersal (Scholin et al. 1995). A paleobiogeographic scenario based on the calculation of a possible molecular clock has been recently proposed to integrate this hypothesis (John et al. 2003b). The high level of genetic diversity detected within the *A. tamarense* complex compared with that recorded among strains of other morphospecies (e.g. *A. minutum*, *A. tamutum*, and *A. ostenfeldii*) from different geographic areas further supports the presence of cryptic species within the *A. tamarense* complex, as shown for other dinoflagellate species (Montresor et al. 2003). Unfortunately, no comparably extensive data sets are available for other *Alexandrium* species, and the assessment of the level of genetic diversity within other morphospecies, as well as the phylogenetic relationships among the different species, are still far from being complete. Until now, the different phylogenies obtained for this genus failed to provide a match with the morphological

characters currently used for species identification. Multiple strains of species that have been identified based on morphological features of their thecal plates indeed group in the same clade. However, for none of these characters (e.g. presence/absence of ventral pore, shape of 1′ or 6″ plates, cyst morphology) is it possible to trace a state that is consistent with the evolutionary picture obtained from molecular analyses. Recently, the grouping of *Alexandrium* species based on the shape of the sulcal posterior plate has been suggested by Usup et al. (2002), but no support for this classification emerged from our data.

Alexandrium tamutum and *A. minutum* are so extremely similar in size and cell outline that they cannot be distinguished during routine cell counts in LM. Both in the Gulfs of Naples and Trieste the two species were detected and identified during investigations carried out on net samples. In the Gulf of Naples, *A. andersoni*, which is another small-sized species, almost indistinguishable from the two mentioned above, has been found (Montresor et al. 1998). Considering that two of the species, *A. andersoni* and *A. minutum*, are PSP producers (Ciminiello et al. 2000, this investigation), their precise identification in monitoring programs becomes a rather important issue. Molecular probes thus represent an extremely promising tool for the rapid and certain HAB species identification (John et al. 2003a). Our results show that the three species are genetically distinct, and the branch lengths leading to this clade would suggest that species-specific rRNA probes based either on SSU or LSU rDNA sequences could be developed for qualitative and quantitative identification.

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