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Annotated list of Scandinavian calcareous dinoflagellates collected in fall 2003

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Abstract: We report nine species of Thoracosphaeraceae (Dinophyceae) from the Western Atlantic Ocean discovered during field work in Sweden and Norway in October 2003. Some of these species are 'cryptic', exhibiting molecular variation in their ITS1 sequences rather than morphological differences. The species list is accompanied by comments on the information necessary to characterize species of calcareous dinoflagellates and on how they were collected with the help of a self-manufactured, rocket-like bore probe.

Zusammenfassung: Wir berichten von neun Arten der Thoracosphaeraceae (Dinophyceae) aus dem Westatlantik, die auf einer Sammelreise im Oktober 2003 nach Schweden und Norwegen identifiziert wurden. Einige dieser Arten sind 'kryptisch' und weisen molekulare Unterschiede zwischen ihren ITS1 Sequenzen, nicht aber bezüglich ihrer Morphologie auf. Die Artenliste ist durch Angaben erweitert, die zur Charakterisierung von Arten Kalkiger Dinoflagellaten notwendig sind und auf welche Weise diese Arten mit Hilfe eines raketenartigen Schwerelots gesammelt wurden.

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Short communication

During the last decade, dinoflagellates producing calcareous cysts (Thoracosphaeraceae or 'calcareous dinoflagellates'; Elbrächter et al. 2008) have been extensively used for climate and environmental reconstruction (Versteegh 1994, Vink et al. 2001, Esper et al. 2004, Meier et al. 2004, Zonneveld et al. 2005). They comprise about 30 described extant species that occur in cold through tropical seas of the world, but diversity and species number appears to be relatively low when compared to the fossil record of approximately 250 species (Streng et al. 2004). The imbalance in the number of extant versus fossil species is not, however, an indication that calcareous dinoflagellates were more abundant in geological times: A number of species whose original description is based on fossil calcareous cysts show stratigraphic ranges to the Pleistocene (e.g., *Bicarinellum*: Versteegh 1993) or are even known from modern sediments (e.g., *Follisdinellum*, *Praecalcigonellum*: Montresor et al. 1998). However, contemporary morphological and molecular investigations on these 'living fossils' have not been undertaken due to the lack of live cultures.

Molecular data support the assumption that there are more than 30 extant species of calcareous dinoflagellates. In many groups of organisms, investigations of the nuclear Internal Transcribed Spacers (ITS) have shown the existence of reproductively isolated units exhibiting molecular, but not morphological, differences ('cryptic species'). This is also true for the *Scrippsiella trochoidea* species complex, nested within Thoracosphaeraceae (D'Onofrio et al. 1999, Montresor et al. 2003), which segregates into at least three major species groups termed STR1, STR2 and STR3 (Gottschling et al. 2005b). The region downstream of helix II found in the secondary structure model of the Internal Transcribed Spacer 1 (Gottschling and Plötner 2004) is very divergent in its primary nucleotide sequence among species. However, this region is intraspecifically invariant, comprising classes of sequence motifs that do not show intermediates between lineages (Fig. 1) and thus might help to determine cryptic species (Gottschling et al. 2005b).

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GeoB 259 SlacA...ACCCACCUUUUGCCU-UGAUCUUGCCUUGGCAAUCUUACUUGCUGACGUGGUUGUCCA-UUUCCUUCUU
GeoB 265 SlacA...ACCCACCUUUUGCCU-UGAUCUUGCCUUGGCAAUCUUACUUGCUGACGUGGUUGUCCA-UUUCCUUCUU
GeoB 288 SlacA...ACCCACCUUUUGCCU-UGAUCUUGCCUUGGCAAUCUUACUUGCUGACGUGGUUGUCCA-UUUCCUUCUU
GeoB 285 SlacB...ACCCACCUUUUGCCU-UGAUCUUGCCUUUGCAAUCUUACUUUCUGAUGUGGUUGUCCA-UUUCCUUCUU
GeoB*253 SlacC...ACCCRCCUUUUGCCU-UGAUCUUGCUUUUGCAAUSUUACUUUCUGAUGUGGUUGUCCA-UUUCCUUCUU
GeoB 277 CAL2....ACCUUCCCUUUGCUCCAU-UGUUGCUUCUGCAGC-UUACAUCUUGAAGUGGUUGUCCACUUUC-UUCUU
GeoB 266 CAL1....ACCCUCC-UUUGCUUCAU-UUUGCCUUCUGCU-CGAUAUACUCCGAAGUGGUUGUCCACUUUCUUUCUU
GeoB 281 CAL1....ACCCUCC-UUUGCUUCAU-UUUGCCUUCUGCU-CGAUAUACUCCGAAGUGGUUGUCCACUUUCUUUCUU
GeoB 306 CAL1....ACCCUCC-UUUGCUUCAU-UUUGCCUUCUGCU-CGAUAUACUCCGAAGUGGUUGUCCACUUUCUUUCUU
GeoB 251 STR2....ACCUCCCUUUUGCCU-UUAUGUUUGCUUUGCAUUCUUAUCUUUCGAAGUGGUUGUCCACUUUC-UUCUU
GeoB*254 STR2....ACCUCCCUUUUGCCU-UUAUGUUUGCUUUGCAUUCUUAUCUUUCGAAGUGGUUGUCCACUUUC-UUCUU
GeoB 263 STR2....ACCUCCCUUUUGCCU-UUAUGUUUGCUUUGCAUUCUUAUCUUUCGAAGUGGUUGUCCACUUUC-UUCUU
GeoB 269 STR2....ACCUCCCUUUUGCCU-UUAUGUUUGCUUUGCAUUCUUAUCUUUCGAAGUGGUUGUCCACUUUC-UUCUU
GeoB 275 STR2....ACCUCCCUUUUGCCU-UUAUGUUUGCUUUGCAUUCUUAUCUUUCGAAGUGGUUGUCCACUUUC-UUCUU
GeoB 289 STR2....ACCUCCCUUUUGCCU-UUAUGUUUGCUUUGCAUUCUUAUCUUUCGAAGUGGUUGUCCACUUUC-UUCUU
GeoB 283 STR3....AACUUCCUUUCGCUUGUGAAGUGUUCUUUGCUAUUUUACCAGUUGAAGUGGUUCUCCCCUUUC-UUCUU
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Fig: 1: Sequence comparison of the ITS1 region between helix II and 5.8S rRNA (see the secondary structure model: Gottschling and Plötner 2004) from selected isolates. Note the absence of intermediates that is indicative for isolated reproductive units.

The existence of cryptic species challenge the listing of species in studies on the ecology, phylogeny or distribution of microorganisms such as the dinoflagellates. Here we report the species composition of (mainly calcareous) dinoflagellates found at localities that have been visited during field work in Sweden and Norway from Oct 24 through Oct 28, 2003 (Tab. 1). We collected sea-water samples with a 20 µm filter and isolated (predominantly peridinoid) motile stages, or we obtained benthos samples at coastal sites and removed cysts for germination. For calcareous dinoflagellates, this procedure to identify species and to establish strains for subsequent investigations was initiated by the work of Wall et al. (1967) and Wall and Dale (1968). The live cultures are held at the University of Bremen (Historische Geologie / Paläontologie) for further studies and for biological conservation.

In order to collect many (benthos) samples in a short period of time, we used a self-manufactured, rocket-like bore probe (Fig. 2). The removable head of the rocket is pointed for penetrating the ground and exhibits an opening for gathering surface sediments. This tool enabled us to conduct very flexible field work and was dropped on a steel cable at any site from bridges or piers in order to collect coastal organisms with neritic developmental stages such as calcareous dinoflagellates.

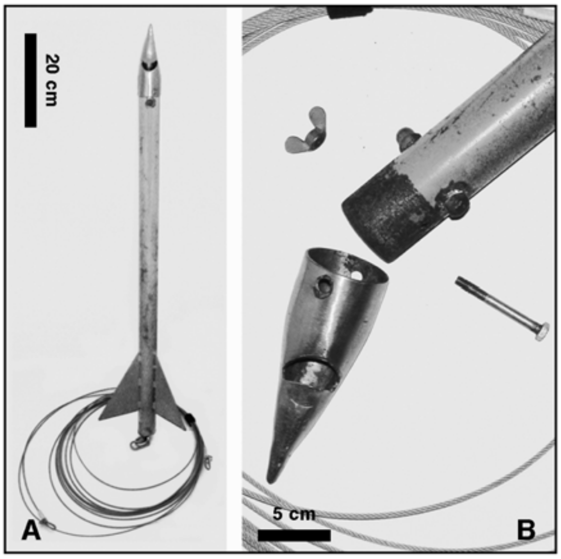

Fig: 2: Rocket-like bore probe for efficient collecting of benthos samples. **A:** General view. **B:** Detail of the removable head.

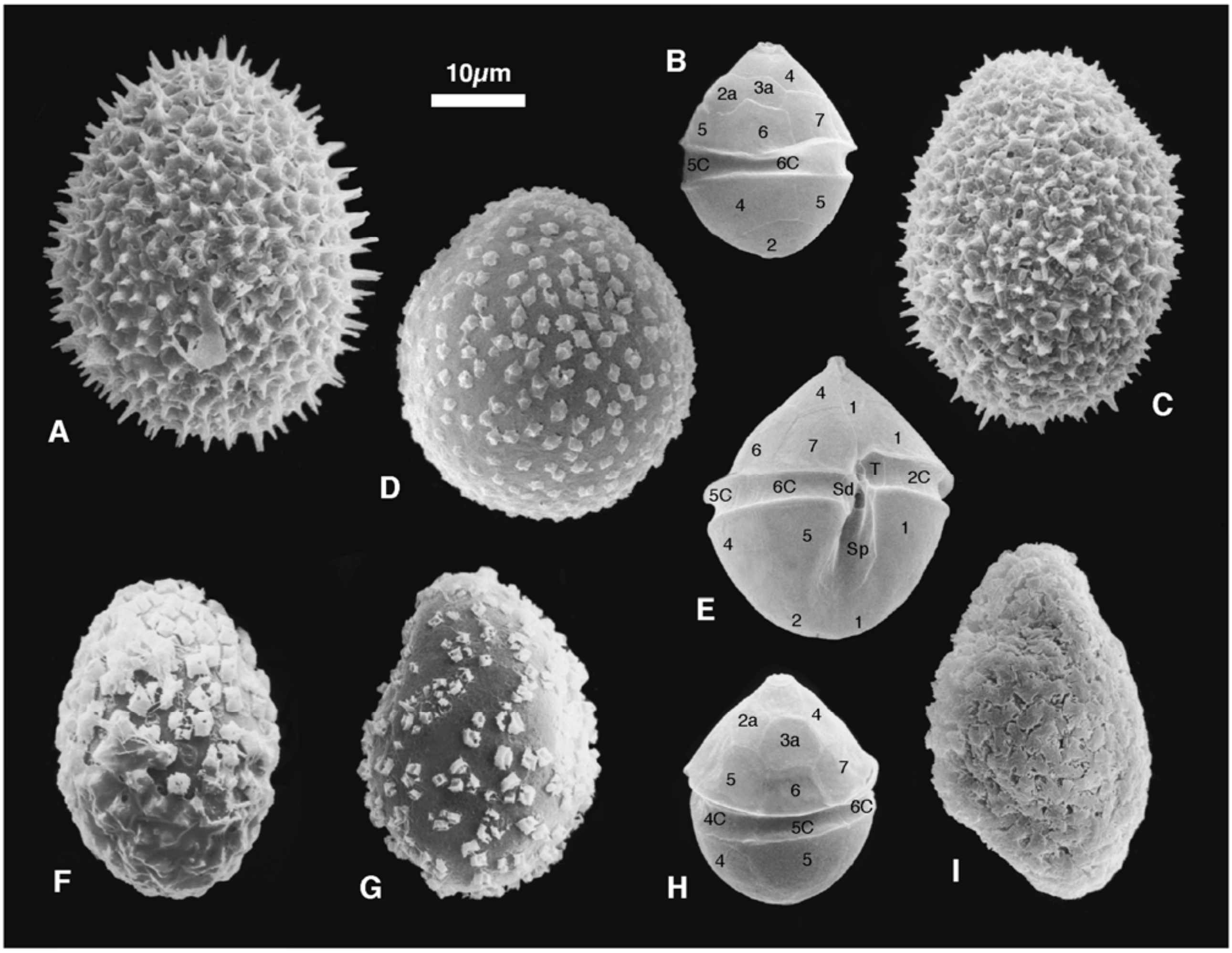

Fig. 3: Different developmental stages of selected strains (thecal plates are indicated). **A-C:** GeoB 256 (*Scrippsiella trochoidea* from the STR2 clade), collected at site SCA0014. **A:** Mature cyst. **- B:** Theka. **- C:** Immature cyst. **- D-E:** GeoB 251 (*Scrippsiella trochoidea* from the STR2 clade), collected at site SCA00001. **D:** Immature cyst. **- E:** Theka. **- F-H:** GeoB 259 (*Scrippsiella lachrymosa* A), collected at site SCA00009. **F:** Mature cyst. **- G:** Immature cyst. **- H:** Theca. **- I:** GeoB 286 (*Scrippsiella lachrymosa* B), mature cyst, collected at site SCA00011.

Four calcareous species of *Scrippsiella* and one species each of *Ensiculifera* and *Pentapharsodinium* have been reported from Western Scandinavia (Dale 1977, 1978, Persson et al. 2000, Godhe et al 2001). Our investigations confirm and expand such findings by mutual comparison of both morphological and molecular data. We found, for example, three (‘cryptic’) *Scrippsiella lachrymosa*-like species and two different species of the *Scrippsiella trochoidea* species complex (Fig. 3). Representatives of the CAL clade nesting within *Scrippsiella sensu lato* (Gottschling et al. 2005a, b) such as *Scrippsiella* cf. *rotunda*, *Scrippsiella donghaiensis* and the yet unnamed species *Scrippsiella* sp. CAL1 have not been reported from Scandinavian waters until now.

From an evolutionary perspective, it is worthy of mention that many calcareous dinoflagellates occur sympatrically at one locality (Tab. 1), and occasionally those species are closely allied (e.g., locality SCA00012 with two *Scrippsiella lachrymosa*-like species). A driving force for speciation has not yet been ascertained for calcareous dinoflagellates. It remains to be determined, to which extent highly diverse coastlines such as the fjords of Scandinavia, with forced isolation mechanisms and their (in geological terms) ephemeral biochorions, are crucial for the diversification of microorganisms with neritic developmental stages, such as calcareous dinoflagellates.

In conclusion, we emphasize the need for not only morphological but also molecular and ecological characterization of (calcareous) dinoflagellates species in future studies, especially when species complexes (such as the *Scrippsiella trochoidea* species complex) are investigated. Such considerations may have also a major impact when they are used in actualistic palaeontology as proxies for reconstructing palaeoecology and palaeoclimate.

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Appendix

Tab. 1: Localities and (mainly calcareous) species of dinoflagellates collected in Oct 2003 in Scandinavia. Abbreviations: CAL1, unnamed species 1 of the CAL clade (*Scrippsiella sensu lato*; Gottschling et al. 2005b); GeoB, Institut für Historische Geologie / Paläontologie (University of Bremen); STR2: 'cryptic' species of the STR2 clade of the *Scrippsiella trochoidea* species complex (Gottschling et al. 2005b); STR3: 'cryptic' species of the STR3 clade of the *Scrippsiella trochoidea* species complex.

Loc. no	country	province	locality	established strains in Bremen	longitude	latitude	salinity	pH	date	remarks
SCA00001	Sweden	Västra Götalands län, Tanum kommun	Havstenssund (harbour), 12km from Tanumshede	GeoB 250 (STR2) GeoB 251 (STR2) GeoB 263 (STR2) GeoB*267 (STR2)	N 58°45'	E 11°11'	27 ‰	6.7	Oct 24	brackish water
SCA00002	Sweden	Västra Götalands län, Strömland kommun	Kosterfjorden near Saltö	GeoB 265 (*S. lachrymosa* A)	N 58°53'	E 11°07'	27 ‰	6.6	Oct 25	open sea
SCA00003	Sweden	Västra Götalands län, Strömland kommun	Kosterfjorden near Daftö	GeoB 266 (*Scrippsiella donghaiensis*) GeoB 305 (*Scrippsiella donghaiensis*) GeoB 306 (*Scrippsiella donghaiensis*) GeoB 307 (*Scrippsiella donghaiensis*) GeoB 309 (*Scrippsiella donghaiensis*)	N 58°54'	E 11°12'	23 ‰	6.7	Oct 25	brackish water
SCA00004	Norway	Sør-Trøndelag, Trondheim kommune	Flakkfjorden, near Flakk (ferry harbour)	–	N 63°24'	E 10°12'	–	–	Oct 26	
SCA00005	Norway	Sør-Trøndelag, Rissa kommune	Flakkfjorden, near Rørvik (ferry harbour)	GeoB 262 (*Prorocentrum* cf. *micans*)	N 63°30'	E 10°08'	27 ‰	7.5	Oct 26	
SCA00006	Norway	Sør-Trøndelag, Rissa kommune	Trondheimsfjorden, Raugbergneset (harbour)	GeoB 269 (STR2) GeoB 272 (*Scrippsiella* spec. CAL1) GeoB 275 (STR2) GeoB 290 (STR2) GeoB 313 (organic-walled dinoflagellate) GeoB 314 (*Gyrodinium* spec.)	N 63°29'	E 9°59'	–	–	Oct 26	low tide sediment, surface sediment
SCA00007	Norway	Sør-Trøndelag, Rissa kommune	Sundsbukta, bridge near Årnset	GeoB 289 (STR2)	N 63°35'	E 9°55'	–	–	Oct 26	low tide sediment
SCA00008	Norway	Sør-Trøndelag, Rissa kommune	Strjørnfjorden, Fevåg-Kai (harbour)	GeoB 277 (*Scrippsiella* spec. CAL1) GeoB 316 (*Gyrodinium* spec.)	N 63°41'	E 9°51'	–	–	Oct 26	
SCA00009	Norway	Sør-Trøndelag, Snillfjord kommune	end of Snillfjorden, Krokstdøra (harbour)	GeoB 259 (*S. lachrymosa* A) GeoB 260 (*Prorocentrum* cf. *micans*) GeoB 280 (*S.* cf. *rotunda*) GeoB 281 (*Scrippsiella donghaiensis*)	N 63°23'	E 9°30'	26 ‰	7.1	Oct 27	
SCA00010	Norway	Sør-Trøndelag, Snillfjord kommune	Åstfjorden, Mjønos (harbour)	GeoB 283 (STR3) GeoB 284 (*Ensiculifera* cf. *imariensis*) GeoB 319 (STR3)	N 63°28'	E 9°25'	–	–	Oct 27	
SCA00011	Norway	Sør-Trøndelag, Frøya island	Titran (fisheri-kai)	GeoB 285 (*S. lachrymosa* B) GeoB 286 (*S. lachrymosa* B) GeoB 322 (*S. lachrymosa* B)	N 63°40'	E 8°18'	31 ‰	7.1	Oct 27	open sea sample
SCA00012	Norway	Sør-Trøndelag, Frøya island	bridge near Måsøyvalen	GeoB*253 (*S. lachrymosa* C) GeoB*254 (STR2) GeoB 288 (*S. lachrymosa* A)	N 63°42'	E 8°30'	–	–	Oct 27	
SCA00013	Norway	Sør-Trøndelag, Snillfjord kommune	Malnes bridge, near Sundan (behind the tunnel to Hitra)	–	N 63°30'	E 9°11'	–	–	Oct 27	
SCA00014	Norway	Vestfold, Sande kommune	Sandebukta, bridge connecting Mørkassel and Bjerkøya	GeoB 255 (STR2) GeoB 256 (STR2) GeoB 257 (STR2) GeoB 261 (*Prorocentrum* cf. *micans*)	N 59°32'	E 10°20'	25 ‰	6.8	Oct 28	