

Appendix 1. List of dinoflagellate cyst species and subspecies

This Appendix alphabetically lists all valid, formally defined dinoflagellate cyst taxa below generic level which are mentioned in this contribution, with full authorial citations. Invalidly published taxa are shown in quotation marks.

Achomosphaera andalusiensis Jan du Chêne 1977

Achomosphaera andalusiensis subsp. *andalusiensis* Jan du Chêne 1977

Achomosphaera andalusiensis subsp. *suttonensis* Head 1997

Achomosphaera argesensis Demetrescu 1989

Achomosphaera callosa Matsuoka 1983

Achomosphaera crassipellis (Deflandre & Cookson 1955) Stover & Evitt 1989

Achomosphaera granulata Mao Shaozhi 1989

Achomosphaera improcera Islam 1983

Achomosphaera ramosasimilis (Yun Hyesu 1981) Londeix et al. 1999

Achomosphaera ramulifera (Deflandre 1937) Evitt 1963

Achomosphaera ramulifera var. *perforata* Davey & Williams 1966

Achomosphaera ramulifera subsp. *perforata* (Davey & Williams 1966) Lentin & Williams 1973

Achomosphaera ramulifera subsp. *ramosasimilis* Yun Hyesu 1981

Achomosphaera sarena Davey & Williams 1966

Baltisphaeridium tripodes Morzadec-Kerfourn 1966

Disphaeria balcanica (Balteş 1971) Norvick 1976

Galeacysta etrusca Corradini & Biffi 1988

Hystrichosphaera bentorii Rossignol 1964

Hystrichosphaera bentorii var. *truncata* Rossignol 1964

Hystrichosphaera bulloidea Deflandre & Cookson 1955

Hystrichosphaera furcata (Ehrenberg 1837) Deflandre 1937

"*Hystrichosphaera furcata*" (Ehrenberg 1837) Wetzel 1933

Hystrichosphaera furcata var. *membranacea* Rossignol 1964

Hystrichosphaera furcata var. *multiplicata* Rossignol 1964
Hystrichosphaera furcata var. *pachyderma* Rossignol 1964
Hystrichosphaera hyperacantha Deflandre & Cookson 1955
Hystrichosphaera membranacea (Rossignol 1964) Wall 1967
Hystrichosphaera mirabilis Rossignol 1964
Hystrichosphaera nodosa Wall 1967
Hystrichosphaera ramosa var. *membranacea* (Rossignol 1964) Davey & Williams 1966
Hystrichosphaera ramosa var. *pachyderma* (Rossignol 1964) Harland & Downie 1969
Hystrichosphaera rubina Rossignol 1962 ex Rossignol 1964
Hystrichosphaera scabrata Wall 1967
Hystrichosphaera tertiaria Eisenack & Gocht 1960
Hystrichosphaera tertiaria var. *obliqua* Wall 1967
Hystrichosphaeridium rubina Rossignol 1962
Impagidinium inaequalis (Wall & Dale in Wall et al. 1973) Londeix et al. 2009
Invertocysta lacrymosa Edwards 1984
Leptodinium churchillii Harland 1968
Pterocysta cruciformis Rochon et al. 2002
Pterodinium cingulatum (Wetzel 1933) Below 1981
Pyxidinospis psilata (Wall & Dale in Wall et al. 1973) Head 1994
Rottnestia ampicavata Dobell & Norris in Harland et al. 1980
Seriliodinium explicatum Eaton 1996
Spiniferites alaskensis Marret et al. 2001 ex Marret in Fensome & Williams 2004
Spiniferites aquilonius Matsuoka & Bujak 1988
Spiniferites asperulus Matsuoka 1983
Spiniferites balcanicus (Balteş 1971) Sütő-Szentai 2000
Spiniferites belerius Reid 1974
Spiniferites bentorii (Rossignol 1964) Wall & Dale 1970

Spiniferites bentorii subsp. *budajenoensis* Sütő-Szentai 1986
“*Spiniferites bentorii* subsp. *coniunctus*” Sütő-Szentai 1990
“*Spiniferites bentorii* subsp. *matraensis*” Sütő-Szentai 1988
Spiniferites bentorii var. *globus* Morzadec-Kerfourn 1979
Spiniferites bentorii subsp. *granulatus* Fuchs & Sütő-Szentai 1991
Spiniferites bentorii subsp. *oblongus* Sütő-Szentai 1986
Spiniferites bentorii subsp. *pannonicus* Sütő-Szentai 1986
“*Spiniferites bentorii* subsp. *piriformis*” Sütő-Szentai 1988
“*Spiniferites bentorii* subsp. *pseudooblongus*” Sütő-Szentai 1983
Spiniferites bulloideus (Deflandre & Cookson 1955) Sarjeant 1970
Spiniferites cingulatus (Wetzel 1933) Sarjeant 1970
Spiniferites crassimuratus (Davey & Williams 1966) Sarjeant 1970
Spiniferites cruciformis Wall & Dale in Wall et al. 1973
Spiniferites delicatus Reid 1974
Spiniferites ellipsoideus Matsuoka 1983
Spiniferites elongatus Reid 1974
Spiniferites falcipediis Warny & Wrenn 1997
Spiniferites firmus Matsuoka 1983
Spiniferites frigidus Harland & Reid in Harland et al. 1980
Spiniferites hainanensis Sun Xuekun & Song Zhichen 1992
Spiniferites hexatypicus Matsuoka 1983
Spiniferites hyperacanthus (Deflandre & Cookson 1955) Cookson & Eisenack 1974
Spiniferites inaequalis Wall & Dale in Wall et al. 1973
Spiniferites lazus Reid 1974
Spiniferites lenzii Below 1982
Spiniferites ludhamensis Head 1996
Spiniferites maisensis Sütő 1994
Spiniferites membranaceus (Rossignol 1964) Sarjeant 1970

Spiniferites multisphaerus Price & Pospelova 2014

Spiniferites nanus Matsuoka 1976

Spiniferites nodosus (Wall 1967) Sarjeant 1970

Spiniferites oblongus (Sütő-Szentai 1986) Soliman & Riding 2017

Spiniferites pachydermus (Rossignol 1964) Reid 1974

Spiniferites pacificus Zhao Yunyun & Morzadec-Kerfourn 1994

Spiniferites perforatus (Davey & Williams 1966) Sarjeant 1970

Spiniferites pseudofurcatus (Klumpp 1953) Sarjeant 1970

Spiniferites pseudofurcatus subsp. *obliquus* (Wall 1967) Lentin & Williams 1973

Spiniferites ramosus (Ehrenberg 1837) Mantell 1854

“*Spiniferites ramosus* subsp. *multiplicatus*” (Rossignol 1964) Lentin & Williams 1973

Spiniferites ramosus subsp. *pachydermus* (Rossignol 1964) Lentin & Williams 1973

Spiniferites ramuliferus (Deflandre 1937) Reid 1974

Spiniferites scabratus (Wall 1967) Sarjeant 1970

Spiniferites rhizophorus Head in Head & Westphal 1999

Spiniferites ristingensis Head 2007

Spiniferites? *rubinus* (Rossignol 1962 ex Rossignol 1964) Sarjeant 1970

Spiniferites septentrionalis Harland 1977

Spiniferites speetonensis Duxbury 1980

Spiniferites spinatus (Song Zhichen in Song Zhichen et al. 1985) Lentin & Williams 1989

Spiniferites splendidus Harland 1979

Spiniferites strictus Matsuoka 1983

Spiniferites? *tripodes* (Morzadec-Kerfourn 1966) Lentin & Williams 1973

Spiniferites validus Sütő-Szentai 1982

Spiniferites virgulaeformis Sütő 1994

“*Subathua balcanica*” (Balteş 1971) Khanna & Singh 1980

Thalassiphora balcanica Balteş 1971

Thalassiphora “*subreticulata*” Fensome & Williams 2005

Appendix 2. List of dinoflagellate species and subspecies

This appendix alphabetically lists all valid, formally defined dinoflagellate species assigned to a motile-defined genus which are mentioned in this contribution, with full authorial citations.

Gonyaulax baltica Ellegaard et al. 2002

Gonyaulax digitale (Pouchet 1883) Kofoid 1911

Gonyaulax elongata (Reid 1974) Ellegaard et al. 2003

Gonyaulax membranacea (Rossignol 1964) Ellegaard et al. 2003

Gonyaulax scrippsae Kofoid 1911

Gonyaulax spinifera (Claparède & Lachmann 1859) Diesing 1866

Lingulodinium polyedra (Stein 1883) Dodge 1989

Protoceratium reticulatum (Claparède & Lachmann 1859) Bütschli 1885

Appendix 3. Supplementary text: round-table discussion on the genus/species/variety concept

KNM: Do we agree to be conservative and keep using the same cyst-based names?

ME: There is no doubt that, if you want the *Spiniferites* classification to be monophyletic, there have to be changes (on the genus level), but doing this now would be premature, since information from molecular data for many central taxa is missing (e.g., *Nematosphaeropsis*); alternatively, the current morphology-based classification and naming (of cysts) can be kept for practical reasons. But we can already discuss preliminary insights.

- LL: It is important that the taxonomy be consistent and practical. Thus 1) everybody uses the same name for the same morphologies, and 2) the relation/common features between species within one single genus are established.
- KNM: But how do you know, when you encounter something new, whether it is a variety of another species or a new species?
- LL: New species are another problem; here we have to be sure that we all agree on the species concept of the existing species.
- GV: A species is a biological concept. When there is no DNA, you only have the morphology to work with. Using or creating subspecies would complicate things; I agree with the suggestion of ME to use DNA on the genus level.
- EM: But what should we do with pre-Quaternary specimens, when we have no possibility to do DNA work or incubation experiments?
- GV: We can work with morphology as well.
- VP: We should not rush with describing new species; evaluate the existing ones and their variations before you are sure you are dealing with something new. It is okay to show morphotypes without giving them explicit names (and just calling them *Spiniferites* type A, B, etc.); once the range of morphological variability is illustrated and better known, a species can be defined.
- ME: A name is only a carrier of information: I agree with the conservative approach.
- BD: So many species have been defined over the years, but some or even many of them have eventually turned out to be useless since either nobody recognises them, or there is no consensus on what they exactly are or how they differ from other species. I prefer that a solid idea of a species exists before it is defined.

- KNM: Let's discuss a practical example. What about *Spiniferites mirabilis* and *Spiniferites hyperacanthus*: should the latter be transferred to a variety of the former?
- BD: There is no scientific basis to do so. Okay, there is perhaps a visual criterion, but formally calling it a variety does not help or change anything for practical determination.
- LL: So we should define morphological concepts, and define what distinguishes them...
- GV: ... and even when DNA shows that *Spiniferites mirabilis* is the same species as *Spiniferites hyperacanthus*, you can still retain the morphotypes!
- KNM: Let's discuss another example: *Hafniasphaera* vs. *Spiniferites multisphaerus* (recently described by Price & Pospelova 2014). There were discussions last year about whether *Spiniferites multisphaerus* should be reclassified as *Hafniasphaera* because of its wall structure. What do we think about this now?
- LL: The problem is semantic; it is a *clear variant*. [LL further noted during draft: probably of *Spiniferites bentorii*.]
- VP: It is a good, identifiable morphotype, so keep it. As shown by Kenneth in his presentation from Rossignol's topotypes, *Spiniferites bentorii* can also show bubbles, so it might be an ecological response, and for that reason should not be transferred to another genus.
- MJH: In this context, I'd like to repeat a comment about the wall structure: I just wanted to underline that wall structure is equally important as other features (such as processes or flanges, for instance).

- VP: Wall structure is an important cyst characteristic, but it also has some ranges of natural variability (e.g., due to environmental influences).
- LL: What is the robustness of a DNA-based genus with respect to morphological features that are high in the hierarchy for determination? In a determination key, generic level comes before the species level.
- ME: So... a “genus” might perhaps not be an existing entity in the real world, but eventually everything should be monophyletic, because clades exist biologically.
- BD: Of course the concept of the genus is important; but I am concerned that we are “destroying” everything that describes the *Spiniferites* genus concept when we try to clean up too much.
- KM: In the biological domain, we focus on the morphology of the theca; for example, the biological genus *Pyrophacus* Stein 1883: *Pyrophacus steinii* (Schiller 1935) Wall & Dale 1971 produces cysts, known as *Tuberculodinium vancampoae* (Rossignol 1962) Wall 1967, and *Pyrophacus horologium* Stein 1883 does not, but both are in the same genus. How can we solve this?
- MJH: I'd like to refer to the genus *Achomosphaera*: this genus was created to split something into a more practical and workable approach, differentiating those cysts from the hundreds of *Spiniferites* species; this was done without the existence of any biological rationale.
- BD: We don't know what the biological meaning is of the morphological separation of *Achomosphaera* and *Spiniferites*.
- KNM: Let's move on, and address *Spiniferites* vs. *Impagidinium* – what do we think of that?

BD: That's very clear: they are distinguished by the presence or absence of processes [KNM adds during draft: but what do we mean by a process? There are several palynological definitions, e.g., Fensome et al. (1993) define it as: "A structure which arises from an external surface and is columnar or spine-like. Processes may be simple or intricately branched and interconnected." Does this then mean that *Impagidinium* has no processes? BD adds during draft: Dale (1996) reported a morphological transition from *Spiniferites* with shorter processes to a few forms that could be assigned to *Impagidinium* with extremely lowered salinities in the inner Baltic, but these are regarded as exceptional ecophenotypic morphotypes of *Spiniferites* that would be readily identified as components of a salinity signal in fossil assemblages.]

GV: But what about *Spiniferites rubinus*?

ME: At the moment this remains an open question because of the molecular data from *Impagidinium caspiense*. Either this one will have to stop being an *Impagidinium* (which is nonetheless a good entity), or the other(s) stop being a *Spiniferites* or *Gonyaulax*.

BD: But it is too soon now to separate them

LL: So we should await more DNA data; it is the same situation with dinosaurs and birds, they are morphological similarities but no DNA data to link them. We could talk of a *Spiniferites*-complex with groups within that combine the known different morphologies, cf. *Impagidinium*, *Achomosphaera*, ...

- BD: I have to add that *Impagidinium* is a paleoecologically important genus all the way back to the Cretaceous; it is a functional group that is very useful (e.g., for basin modeling, see for instance Dale et al. 2005).
- KZ: We can describe the variation within a species, keep the names, and eventually connect the morphological end-members.
- VT: I have to underline that the separation of *Spiniferites*, *Achomosphaera* and *Impagidinium* was the utmost practical aspect until now. [With respect to the potential role of molecular data, he points out that in the seventies, attempts were made to clean up the genus *Impagidinium*. They looked at samples and specimens from all kinds of environments (also “stressful” environments), and still the separation of the genus was retained.]
- MB: I would propose to call all the cysts “cyst of *G. baltica*”, and describe the morphotypes separately. Then *I. caspiense* is a junior synonym; we keep the names in function of ecological significance.
- ME: But we probably still will have to come up with a new genus name, with *G. digitale* (*B. tepikiense*) currently sitting bang in the middle of the phylogenetic tree...
- BD: ... but that is because the genus is *Gonyaulax*, not *Spiniferites*, from a biological point of view!
- LL: Are we certain that the tabulation from the cyst and theca are identical? How does the tabulation of *Gonyaulax* compare with that of *Impagidinium*?
- BD: This has surely already been looked at; it is just that *Impagidinium* cysts do not always show full tabulation.

MJH: Well, *Impagidinium sphaericum* does, and (in general?) the tabulation of the cyst is not always fully representative of that of the theca.

KNM: Another thing, what do we think about the species boundaries? For instance, what do we consider a variety of a species?

LL: To formally accept varieties would go against history: Lentin & Williams (1973 etc.) created a lot of subspecies by consistently transferring forms and varieties to the subspecies level. They stopped doing that at a certain point because of some criticism, and from then on kept morphotypes at the level of the original description.

MJH: Forms and varieties were raised in rank to subspecies.

KZ: We should think of continuums with end members.

MJH: Using “forma” is as conservative as you can get; it is very objective.

ME: I agree, but I wonder what we should do with ecophenotypes?

BD: My feeling is that new subdivisions are not needed, only a consensus on the variability of a species; we don’t always know if a morphotype is indeed an ecophenotype.

GV: The use of “subspecies” has important biological implications.

KNM: Again, a concrete example: would it be okay to use a “forma *intergonalis*” for a morphological variant bearing consistent intergonal processes?

BD: If we have things in between...no.

ME: Yes, if there is information in it [ME notes during draft that she meant if dividing the species gives more information than merely the morphological difference, for example, if there are geographical or ecological or temporal

differences between the forms – so that use of both names will carry this information].

BD: I repeat that we should only have to document the variability within a species, but that one of the problems is that many people have only looked in one region and thus likely have not documented the full range of morphological variability; however, variation is unavoidable. We need global information to address variation.

KNM: For example, *Spiniferites bentorii* with two intergonals at every suture: forma *intergonalis* or not?

LL: Depends whether this is a consistent feature? Often this is a subjective interpretation.

Several remarks are made ... but the overall feeling is that this would be problematic; something has to be a consistent feature on several specimens in order to constitute a forma.

ME: If it is only found within your own research area, you should call it a morphotype. If it is found in many places eventually it can be called more officially.

VP: I would agree with this approach.

KNM: What about the species concept; when should something be a species, when a forma?

BD: There is no answer to that.

LL: Proposes to follow Fensome & Williams (2004), otherwise known as Dinoflaj2.

BD: It's almost a gut feeling; don't make too many species.

- KZ: I'd propose to use "cyst of" when the biological affinity is known.
- GV: There are many species concepts; concerning the morphological species concept.
[He makes an analogy with plants where forma is mostly used to describe something aberrant and rare.]
- KNM: So a species should have enough morphological characters to be distinguishable from other species?
- BD: Has always been done so.
- VP: Stresses again that she feels that the range of morphological variability should be well documented before publishing a new species. Colleagues should be consulted beforehand, sending around pictures etc.
- VT: I'd like to give an example from tropical pollen research to pick up on Vera's remark: publications from the sixties only showed pictures with informal names (A, B, C), later it was decided what the key morphological features were, and only then species were described. I think this is a very clear and neat way of working.
- BD: In the early days it was done like this; gradually information of different people got together and more reasonable descriptions were the product of this. At this point I also wonder whether we will formalise our recommendations.
- KNM: I think we should do that.

The discussion moves on to the topic of double nomenclature. ME gives a brief lecture on how to interpret a molecular phylogenetic tree.

MJH: I'd like to pick up on the fact that from the molecular tree, the genus *Spiniferites* appears to be polyphyletic. So, would you have to redefine the genus, and what would you have to do with all the species that you have to move out, some going back as far as the Jurassic?

BD: This is a genetic tree and you therefore do not necessarily have to change the morphology-based concepts.

PG: I would propose to attempt to make a tree based on morphological concepts (cladistics) and hold it up against the molecular tree

VP: But... the specimen that you pick for molecular analysis is already a morphology-based interpretation because of the name you give to it; it is hard to disconnect morphology-based and DNA-based taxonomy.

LL: Should we have genetic data from the same species from more varied environments and regions, and particularly from the *Impagidinium* species?

The discussion continues, but there appears to be some consensus on two main points: 1) we obviously need more different specimens for DNA work; 2) dual nomenclature can be advantageous from a practical point of view.

ME: Yes, the tree is still suffering from reduced taxon sampling; now on the dual nomenclature; we want to get monophyletic groups; if we want to call it all *Gonyaulax* (which is definitely monophyletic) there are different options of renaming species; or rename everything to cyst of *Gonyaulax*? That would mean minimal confusion...

- GV: ...unless you go one step up in the tree, and making all of it *Gonyaulax*
- KNM: All of this relies on the reliability of the molecular data.
- ME: Apart from everything, this is a good tree... very robust.
- VP: Take molecular data for *Impagidinium* from more typical environments.
- ME: Yes, this species included is not the most typical one.
- ME: The problem with defining new genera from the molecular tree is that we would have to find common morphological features, which seems very unlikely (for instance: *Bitectatodinium tepikiense* among *Spiniferites*...).
- VP: It is very important to add DNA data from specimens from “typical” environments that constitute clear end-members, to constitute the fundamentals of the molecular tree.
- BD: I feel that 1) in the end, there will be no real change in the paleontological taxonomy, and 2) DNA work is “gonna take off” in the near future; it is an excellent tool and provides crucial support for those working with cysts, but it should probably not have any real consequences for paleontological, biostratigraphical cyst-based work; what will change is the support, the framework of our system; we will get ever better data and trees but we do not have to bother about the implications to the names of the species.
- MJH: I'd like to address a practical issue; there is only one holotype which biologists prefer to be the motile cell; if you describe the holotype as a motile cell, we don't have a type for the cyst, and the theca will not be preserved except for photographs; the ideal thing would be to take a wild cyst and let it hatch and let it be the holotype; but, this is practically hard to do. Furthermore, this holotype

cannot be preserved in the case of a DNA-based species; the photograph becomes the holotype, but you can never go back to the actual specimen; as such, DNA cannot serve as a base for taxonomy.

[AR remarks during draft that he does not agree: a thecate stage excysted from cysts can be mounted in "permanent" slides made with glycerine jelly; KNM agrees and furthermore adds that motile stages can also be "permanently" preserved on goldcoated SEM stubs or as cultures in culture collections; the latter also enables the preservation of DNA. SR agrees and considers that we should even recommend that, whenever possible, a description of a new species should be based on a combination of cyst morphology ("kept" in a permanent slide) and vegetative stage information – morphology and DNA from excysted stages; goldcoated SEM stubs also preserve DNA, and cells kept in culture can be fixed with for instance Lugols acid in order to preserve the motile cells and their DNA for years – which is safer and in some ways better than maintaining a culture for many years, to avoid any changes introduced by lab selection and mutations. HG adds that cells can be frozen to preserve their DNA for many years].

KM: Nonetheless, DNA is already the backbone of taxonomy in other areas.

ME: Molecular data is ok, but not enough to define taxa; morphology is always needed; furthermore, we should evaluate what characteristics determine the branching; interpret the branch lengths to see evolutionary history, including information from the fossil record. There are practical reasons to conserve dual nomenclature.

BD: Dale (1983) still provides the best reference on dual nomenclature.

