

ASCIDIAN NEWS*

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I greatly enjoyed participating in teaching a two week ascidian course at Nagoya University's Sugashima Marine Lab from the end of June to July 10, and then attended the Intl. Tunicata meeting in Aomori, Japan from July 13-17.

This issue is the second for my 40th year of compiling Ascidian News. I would greatly appreciate hearing from you whether you still find it useful and interesting. There are **93** New Publications listed at the end of this issue.

***Ascidian News is not part of the scientific literature and should not be cited as such.**

NEWS AND VIEWS

1. *Ciona intestinalis* now shown to be 2 separate species. Because so many researchers work on *Ciona intestinalis*, and so many papers are published on this species, I draw your attention to 2 new publications showing at last that *Ciona intestinalis* A and B are different species and designating the correct names to be used in all future publications:

Brunetti, R., Gissi, C., Pennati, R., Caicci, F., Gasparini, F. and Manni, L. 2015. Morphological evidence indicates that *Ciona intestinalis* (Tunicata, Ascidiacea) type A and type B are different species. *Journal of Zool. Systematics & Evolutionary Research* 53 (3): 186–193. [Type A is now designated *C. robusta*; type B retains the name *C. intestinalis*.]

The second new publication describes larval differences between the two species: Pennati, R., Ficetola, G. F., Brunetti, R., Caicci, F., Gasparini, F., Griggio, F., Sato, A., Stach, T., Kaul-Strehlow, S., Gissi, C. and Manni, L. 2015. Morphological differences between larvae of the *Ciona intestinalis* species complex: hints for a valid taxonomic definition of distinct species. *PLoS One* 10(5): 1-22.

See the Work in Progress section for an important detailed report of the 8th Intl. Tunicata meeting Round Table discussion: Taxonomy of *Ciona* spp. and Suggestions for species designations in publications on *Ciona intestinalis* and/or *Ciona robusta*.

2. The 9th Intl. Conference on Marine Bioinvasions will be held in Sydney, Australia January 19-21, 2016. For more information, go to www.marinebioinvasions.info or contact Dr. Emma Johnston: e.johnston@unsw.edu.au.

3. The 8th Intl. Tunicata meeting was held July 13-17, 2015 in Aomori city, Japan. The full program can be found at <http://tunicatameeting.info/Aomori2015/?q=program>. Click on the links for Oral presentations pdf and Poster presentations pdf to view all titles.

4. From Joana Dias (Joana.Dias@fish.wa.gov.au).

I am a researcher at the Western Australia Department of Fisheries working on the population genetics of the marine invasive sea squirt *Didemnum perlucidum*. This species is a tropical/temperate white colonial ascidian and can be found fouling artificial structures in ports, marinas and aquaculture worldwide in warm waters. Its native range is however unknown and finding it would help us better understand its invasive potential. If you work with marine invasive species, particularly in a tropical country, or know someone who does and think you could help finding if it is in your area, please drop me an email. I am happy to send you some pictures.

Thank you, any information will be most appreciated!

Given the widespread low COI diversity of this species, we have developed microsatellite markers to further study its introduction in Australia. The publication has been accepted and is in press:

Dias PJ, Simpson T, Hitchen Y, Lukehurst S, Snow M and Kennington WJ (in press) Isolation and characterization of 17 polymorphic microsatellite loci for the widespread ascidian *Didemnum perlucidum* (Tunicata, Ascidiacea). *Management of Biological Invasions Short Communication*.

5. Anna Di Gregorio (adg13@nyu.edu) (NYU College of Dentistry) would like to announce that she is celebrating 20 years of publications in ascidian biology (Di Gregorio et al., 1995 to Thompson and Di Gregorio, 2015) and has an upcoming publication from the lab by Diana Jose'-Edwards et al. Congratulations Anna!

6. From Elisabetta Tosti (tosti@szn.it), Stazione Zoologica, Naples.

A new paper by Gallo and Tosti includes the following cartoon she thought would be of interest and amusement to AN readers.

Ciona intestinalis: from endangered species to top model

Gallo & Tosti, 2015. (artwork by Fiammetta Formisano) [see New Publications section for the complete citation.]



Elisabetta writes that in March 2016 she will have worked 40 years at the Stazione Zoologica in Naples. Congratulations Elisabetta!

WORK IN PROGRESS

1. 8th International Tunicata meeting, Aomori, Japan July 13-17, 2015 Round Table Summary: Taxonomy of *Ciona* spp.

At the conclusion of the Tunicata meeting a round table discussion “Taxonomy of *Ciona* spp.” was organized by Lucia Manni (University of Padova, Italy) (lmanni@civ.bio.unipd.it) together with Fabio Gasparini (University of Padova, Italy), Carmela Gissi (University of Milan, Italy), Thomas Stach (Humboldt-Universität zu Berlin, Germany) and Ken Hastings (McGill Univ., Canada). The following document was prepared from that discussion. The meeting participants were invited to:

- become aware that *Ciona intestinalis* type A is *Ciona robusta* Hoshino & Tokioka, 1967, and *Ciona intestinalis* type B is *Ciona intestinalis* (Linnaeus, 1767) (see Brunetti et al., 2015; Pennati et al., 2015)
- learn how to distinguish the two species looking at adults and/or larvae;
- verify if information already in public databases belongs to *C.intestinalis* or to *C. robusta*
- discuss the opportunity to update the public tunicate databases with the correct species names.

Presentation on species nomenclature and diagnostic criteria

Nomenclature

Ciona intestinalis type A is assigned to *Ciona robusta* Hoshino and Tokioka, 1967 because both possess tubercular prominences on the tunic.

Ciona intestinalis type B is assigned to *Ciona intestinalis* (Linnaeus, 1767) because:

- 1) *Ciona intestinalis* type B is common on North Atlantic coasts (Suzuki et al. 2005; Caputi et al. 2007; Nydam and Harrison 2007)
- 2) *Ciona intestinalis* (L.) was first described – as *Ascidia intestinalis* – from the Northern European Seas (Linnaeus’ “Oceano europaeo” Syst. Nat. 1791. Gmelin edition page 3123)
- 3) *Ciona intestinalis* (L.) is universally recognized to correspond to Millar’s description (1953) (referred to animals sampled in British waters).

Ciona intestinalis sensu Hoshino and Nishikawa (1985) included both *Ciona intestinalis* type A and type B (see Table 1).

Diagnostic criteria

Researchers were invited to examine adult individuals in order to verify which species they are studying, using a dissection microscope with transmitted and/or reflected light, and isolating the tunic if necessary.

Tubercular prominences on the tunic are papilla-shaped or elongated protuberances. They are distributed along the whole body and often more conspicuous around the siphons, where they prevalently are arranged in longitudinal rows.

Late swimming larva (stage 29 according to the FABA2 database - <http://chordate.bpni.bio.keio.ac.jp/faba2/2.2/top.html> - or stage 2 according to Chiba et al., 2004; 24 h post-fertilization at 18°C) belonging to the two species can be discriminated. The larvae of *C. intestinalis* have a longer pre-oral lobe, a longer and relatively narrower total body length, and a shorter ocellus-tail distance than larvae of *C. robusta*. Researchers can successfully apply two different discriminant functions based on four or two larval morphometric parameters to discriminate larvae belonging to the two species (Pennati et al., 2015).

Points arising during round table discussion

The discussion was positive and collaborative although the issue was recognized as a complex one, with different significance for different disciplines

It was stressed that the two taxa are genetically divergent. They display deep molecular divergence (14% of homologous codons encoding identical amino acid residues are distinct synonyms in the two species (Roux et al.2013)). In addition, an extensive population survey showed that only a single F1-hybrid and no backcrossed individual were identified in nature in the only area where the two types are presently known to co-occur (Bouchemousse et al. (2015) and talk by Frederique Viard). While acknowledging that species definition and delimitation is complex because amongst other reasons speciation is a gradual process, these results, combined with significant morphological differences, strongly support the assignment of type A and type B to distinct species following phylogenetic and morphological criteria (Frederique Viard from the Station Biologique de Roscoff; Xavier Turon from CSIC Blanes).

Molecular and developmental biologists attending the meeting were worried that the Tunicate Community may be seen as lacking scientific rigor when they had to admit that the genome of *C. intestinalis* published in 2002 (Dehal et al., 2002) now turned out to be from *C. robusta*. Moreover, they were worried that the split of the “previous” single *C. intestinalis* taxon into two distinct species could produce chaos in the interpretation of the previous literature.

Taxonomists and evolutionary biologists, used to name changes, argued that on the contrary the community could be seen as lax only if it would not follow the latest scientific evidence. Systematics is a dynamic field and species are working hypothesis. Pointing out the 250 years of tradition leading to a set of rules – The International Code of Zoological Nomenclature (ICZN, 4ed) – both parties discovered in their debate that these rules, although formalistic and legalistic, were designed to secure the main common goal: enabling science to progress and restore order in debated issues. Therefore the taxonomically valid name of *C. robusta* has to be used instead of “*C. intestinalis* type A” from now on. This will allow future

scientists to understand which species had been used in the respective molecular or developmental research. In particular, it was advised that the collection site (or resources) and the collection dates of the animals should be stated in the Materials and Methods in all future reports. The community should also consider that the two species are possibly distributed sympatrically even in regions other than the English Channel, and that the distribution of the two species can change during time.

Several proposals were put forward and discussed:

Researchers who deposited their *Ciona* sequences in public databases (GenBank, ENA, Ensembl, JGI Genome Browser, UCSC Genome Browser, etc.) are invited to contact the database managers and to change the species names if they are now seen to be in error. In addition, the community suggested that the *Ciona* species names and taxonomy should be updated in specialized reference databases, such as WoRMS (World Register of Marine Species) (suggestion of Xavier Turon) and possibly also in ANISEED, because it reports not only genomic sequences but also anatomical and gene expression data.

In terms of citations, especially in the imminent future, where there is potential for confusion, several clear and transparent ways of citation are suggested:

- *Ciona robusta* Hoshino and Tokioka, 1967. Formerly *Ciona intestinalis* type A (see Brunetti et al., 2015; Pennati et al., 2015) (suggested by Gretchen Lambert from University of Washington Friday Harbor Labs, USA)
- *Ciona robusta* (= former *C. intestinalis* type A *sensu* Nydam and Harrison, 2007) (suggested by Euichi Hirose from University of Ryukyus, Japan)
- *Ciona intestinalis* (Linnaeus, 1767), according to Brunetti et al. (2015) and Pennati et al. (2015). Formerly *Ciona intestinalis* type B (suggested by G. Lambert).

Table 1. Summary of changes occurred in the taxonomy of *Ciona* sp. (by E. Hirose).

Species name	<i>Ciona intestinalis</i> (Linnaeus, 1767)	<i>Ciona robusta</i> Hoshino et Tokioka, 1967	<i>Ciona savignyi</i> Herdman, 1882
Type locality	Oceano Europaeo	Onagawa, Miyagi, Japan	Kobe, Hyogo, Japan
Type specimen	Original type was missing. Neotype is deposited in Natural History Museum in Venice	Syntype specimens were deposited in Seto Marine Biol. Lab., Kyoto Univ.	British Museum of natural Science
Linnaeus 1767	<i>Ciona intestinalis</i>		
Hoshino et Tokioka, 1967	<i>Ciona intestinalis</i>	<i>Ciona robusta</i>	<i>Ciona intestinalis</i>
Hoshina and Nishikawa, 1985	<i>Ciona intestinalis</i>	<i>Ciona intestinalis</i>	<i>Ciona savignyi</i>
Brunetti et al., 2015	<i>Ciona intestinalis</i>	<i>Ciona robusta</i>	<i>Ciona savignyi</i>

*This table does not included synonyms that are invalid at present.

Suggestions for species designations in publications on *Ciona intestinalis* and/or *Ciona robusta*

Overview

The following suggestions regarding Title and Abstract are meant to minimize confusion in the non-specialist scientific reader during the transitional period in which the term "*Ciona robusta*" is first coming into common use. In a few years, when the scientific public has become familiar with the *intestinalis/robusta* split, these suggestions will have lost their importance. However, the suggestions for the Methods may have a more permanent validity.

The suggestions for the Methods section should be considered as "minimal requirements". We suggest to detail as much as possible the methodology used for the morphological and/or the molecular characterization of the specimens, i.e. reporting which genes(s), sequence(s) or restriction fragment(s) were analyzed; which larval characters were measured; if the presence/absence of tubercles was investigated with a dissection microscope with transmitted and/or reflected light; etc.

Rationale

The rationale is that during the transitional period the new and unfamiliar name *Ciona robusta* will not appear in the Title or Abstract except in the company of the familiar name *Ciona intestinalis*. First mention of *Ciona robusta* in Abstracts will always be linked to "formerly *Ciona intestinalis* type A". For logical coherence and precision, in the Title or Abstract the name *Ciona intestinalis* should be linked to "formerly *Ciona intestinalis* type B". Use of the generic term "*Ciona*" in the Title can in many cases be a useful approach to maintain simplicity when the species complexity is not especially relevant.

Suggested species designations

Your study of *Ciona intestinalis* and/or *robusta* will likely fall into one of the following 6 classes. A seventh class, at the end, includes studies that also involve an additional *Ciona* species beyond *intestinalis/robusta*, e.g. *Ciona savignyi*.

1) It is probable, based on the species geographic ranges, that all the animals studied were *Ciona intestinalis*

Title: say "*Ciona*"

Abstract: say "*Ciona intestinalis* (formerly *Ciona intestinalis* type B)"

Methods (under Animals): In 2015 it was recognized that *Ciona intestinalis* included two distinct species, *Ciona intestinalis* (formerly *Ciona intestinalis* type B) and *Ciona robusta* (formerly *Ciona intestinalis* type A). The animals used in the present study were collected in (collection date) at (name sites) at which only *Ciona intestinalis* is known to occur (range citation). Species-defining morphological or molecular characters were not assessed, but based on current knowledge of distribution we assume that the animals studied were *Ciona intestinalis*.

2) It is known, based on morphological or molecular criteria, that all the animals studied were *Ciona intestinalis*

Title: say "*Ciona*"

Abstract: say "*Ciona intestinalis* (formerly *Ciona intestinalis* type B)"

Methods (under Animals): In 2015 it was recognized that *Ciona intestinalis* included two distinct species, *Ciona intestinalis* (formerly *Ciona intestinalis* type B) and *Ciona robusta* (formerly *Ciona intestinalis* type A). The animals used in the present study were collected in (collection date) at (name sites) at which only *Ciona intestinalis* is known to occur (range citation). Species-defining morphological or molecular characters were assessed (name the characters), which confirmed the animals studied were *Ciona intestinalis*.

3) It is probable, based on the species geographic ranges, that all the animals studied were *Ciona robusta*.

Title: say "*Ciona*"

Abstract: say "*Ciona robusta* (formerly *Ciona intestinalis* type A)"

Methods (under Animals): In 2015 it was recognized that *Ciona intestinalis* included two distinct species, *Ciona intestinalis* (formerly *Ciona intestinalis* type B) and *Ciona robusta* (formerly *Ciona intestinalis* type A). The animals used in the present study were collected in (collection date) at (name sites) at which only *Ciona robusta* is known to occur (range citation). Species-defining morphological or molecular characters were not assessed, but based on current knowledge of distribution we assume that the animals studied were *Ciona robusta*.

4) It is known, based on morphological or molecular criteria, that all the animals studied were *Ciona robusta*.

Title: say "*Ciona*"

Abstract: say "*Ciona robusta* (formerly *Ciona intestinalis* type A)"

Methods (under Animals): In 2015 it was recognized that *Ciona intestinalis* included two distinct species, *Ciona intestinalis* (formerly *Ciona intestinalis* type B) and *Ciona robusta* (formerly *Ciona intestinalis* type A). The animals used in the present study were collected in (collection date) at (name sites) at which only *Ciona robusta* is known to occur (range citation). Species-defining morphological or molecular characters were assessed (name the characters), which confirmed the animals studied were *Ciona robusta*.

5) It is possible, based on the species geographic ranges, that the animals studied included both *Ciona intestinalis* and *Ciona robusta*.

Title: say "*Ciona*"

Abstract: say "*Ciona intestinalis* (formerly *Ciona intestinalis* type B)" and "*Ciona robusta* (formerly *Ciona intestinalis* type A)"

Methods (under Animals): In 2015 it was recognized that *Ciona intestinalis* included two distinct species, *Ciona intestinalis* (formerly *Ciona intestinalis* type B) and *Ciona robusta* (formerly *Ciona intestinalis* type A). The animals used in the present study were collected in (collection date) at (name sites) at which both species are known to occur (range citation). Species-defining morphological or molecular characters were not assessed, but based on current knowledge of distribution we assume that the animals studied may have included both species.

6) It is known, based on morphological or molecular criteria, that some of the animals studied were *Ciona intestinalis* and some were *Ciona robusta*.

Within this class of studies there will be two major subclasses.

6A. Studies reporting differences between the species, or studies in which possible species differences were looked for and not found.

Title: say "*Ciona intestinalis* and "*Ciona robusta*"

Abstract: say "*Ciona intestinalis* (formerly *Ciona intestinalis* type B) and "*Ciona robusta* (formerly *Ciona intestinalis* type A)"

Methods (under Animals): In 2015 it was recognized that *Ciona intestinalis* included two distinct species, *Ciona intestinalis* (formerly *Ciona intestinalis* type B) and *Ciona robusta* (formerly *Ciona intestinalis* type A). The animals used in the present study were collected in (collection date) at (name sites) at which both species occur (or at distinct sites for each species). Species-defining morphological or molecular characters (name the characters) were assessed.

6B. Studies in which it was not an important goal to compare the species, but in which they were both assessed incidentally and no difference was found.

Title: say "*Ciona*".

Abstract: say "*Ciona intestinalis* (formerly *Ciona intestinalis* type B) " and "*Ciona robusta* (formerly *Ciona intestinalis* type A)"

Methods (under Animals): In 2015 it was recognized that *Ciona intestinalis* included two distinct species, *Ciona intestinalis* (formerly *Ciona intestinalis* type B) and *Ciona robusta* (formerly *Ciona intestinalis* type A). The animals used in the present study were collected in (collection date) at (name sites) at which both species occur (or at distinct sites for each species). Species-defining morphological or molecular characters (name the characters) were assessed.

[Comment by Carmela Gissi: In my opinion the distinction in two subclasses based on the goal of the study is not necessary: I suggest to use the sentences of case 6A in publications of both subclasses, and to delete in this file all sentences concerning "6B".]

7) Studies including an additional *Ciona* species, e.g. *Ciona savignyi*.

Title: The suggestion in classes 1 - 5 and 6B to use the generic "*Ciona*" in the title would not be appropriate in class 7. Having to name, e.g., *Ciona savignyi* in the title would force you to supply a species name for your *Ciona intestinalis/robusta* material in the title. In this case say "*Ciona intestinalis* (formerly *Ciona intestinalis* type B)" and/or "*Ciona robusta* (formerly *Ciona intestinalis* type A)", as appropriate.

Abstract: say "*Ciona intestinalis* (formerly *Ciona intestinalis* type B) and/or "*Ciona robusta* (formerly *Ciona intestinalis* type A)" as appropriate.

Methods (under Animals): In 2015 it was recognized that *Ciona intestinalis* included two distinct species, *Ciona intestinalis* (formerly *Ciona intestinalis* type B) and *Ciona robusta* (formerly *Ciona intestinalis* type A). *Structure the remainder of the Methods along the lines indicated above for one of classes 1 - 6, whichever is most relevant.*

Literature (* means reference includes range information, of use in helping to determine which species was actually used)

*Bouchemousse S , Lévêque L, Dubois G, Viard F (2015) Co-occurrence and reproductive synchrony do not ensure hybridization between an alien tunicate and its interfertile native

congener. *Evol Ecol*. IN PRESS. First online: 09 August 2015. doi:10.1007/s10682-015-9788-1.

- *Brunetti R, Gissi C, Pennati R, Caicci F, Gasparini F, Manni L (2015) Morphological evidence that the molecularly determined *Ciona intestinalis* type A and type B are different species: *Ciona robusta* and *Ciona intestinalis*. *J Zoolog Syst Evol Res*. 53:186-193.
- *Caputi L, Andreakis N, Mastrototaro F, Cirino P, Vassillo M, Sordino P (2007) Cryptic speciation in a model invertebrate chordate. *Proc Natl Acad Sci USA* 104:9364-9369.
- Chiba S, Sasaki A, Nakayama A, Takamura K, Satoh N (2004) Development of *Ciona intestinalis* juveniles (through 2nd ascidian stage). *Zoolog Sci* 21: 285–298.
- Dehal P, Satou Y, Campbell RK, Chapman J, Degnan B, et al. (2002) The draft genome of *Ciona intestinalis*: insights into chordate and vertebrate origins. *Science* 298:2157-2167.
- *Hoshino ZI, Tokioka T (1967) An unusually robust *Ciona* from the northeastern coast of Honsyu Island, Japan. *Publ Seto Mar Biol Lab* 15:275-290.
- ICZN. *International Code of Zoological Nomenclature*. 4 ed., (The International Trust for Zoological Nomenclature, 1999).
- Linnaeus C (1767) *Systema naturae*. Editio duodecima. Holmiae, Stockholm.
- Linnaeus C (1791) *Systema Naturae*. Editio decima tertia (“Gmelin edition”). Lipsiae, Leipzig.
- *Millar RH (1953) *Ciona*. Colman, J. S. ed. Liverpool University Press, Liverpool.
- *Nydam ML, Harrison RG (2007) Genealogical relationships within and among shallow-water *Ciona* species (Asciacea). *Mar Biol* 151:1839-1847.
- *Nydam ML, Harrison RG (2011) Introgression despite substantial divergence in a broadcast spawning marine invertebrate. *Evolution* 65: 429-442
- *Pennati R, Ficetola GF, Brunetti R, Caicci F, Gasparini F, Griggio F, Sato A, Stach T, Kaul-Strehlow S, Gissi C, Manni L (2015) Morphological differences between larvae of the *Ciona intestinalis* species complex: hints for a valid taxonomic definition of distinct species. *PLoS ONE* 10:e0122879.
- *Roux C, Tsagkogeorga G, Bierne N, Galtier N (2013) Crossing the species barrier: genomic hotspots of introgression between two highly divergent *Ciona intestinalis* species. *Mol Biol Evol* 30: 1574-158.
- *Suzuki MM, Nishikawa T, Bird A (2005) Genomic approaches reveal unexpected genetic divergence within *Ciona intestinalis*. *J Mol Evol* 61: 627–635.
- *Zhan A, Macisaac HJ, Cristescu ME (2010) Invasion genetics of the *Ciona intestinalis* species complex: from regional endemism to global homogeneity. *Molecular Ecology* 19: 4678-4694.

2. From Delphine Dauga (contact@aniseed.cnrs.fr)

After 3 years of refactoring the ANISEED database, its user interfaces, and the data curation system, we are happy to announce the publication in the 2016 Database issue of *Nucleic Acid Research* the improvement and update of ANISEED. [Brozovic, Martin, Dantec, Dauga et al. *NAR 2016 Database issue*, In press—see *New Publications* section].

In this article, we report the development of the system since its initial publication in 2010:

- A new and more adapted database schema
- An improved and enriched formal description of the embryonic development of *Ciona*, *Phallusia*, *Halocynthia* and *Molgula* species, and of budding in *Botryllus*.
- The genomes of nine ascidian species can be explored via dedicated genome browsers, and searched by Blast.

- A full functional gene annotation, anatomical ontologies and some gene expression data for the six species with highest quality genomes are now available.

ANISEED is publicly available at: <http://www.aniseed.cnrs.fr> .

During the refactoring process we had to stop inserting expression data from published articles, and the last article of the 189 articles currently included in the database was entered in June 2011. Since then, however, many articles dealing with the molecular developmental biology of *Ciona* have been published, a number which vastly exceeds our current biocuration ability. We are therefore looking for volunteers to enter data from their own papers, or from other papers they know well in *Ciona*, but also in *Phallusia*, *Halocynthia* and *Botryllus*.

We are aware that entering data is a time consuming task. Yet, there are many reasons to volunteer:

- At a time when reading (and remembering) all papers in a field is becoming difficult, ANISEED will make your work more visible and more accessible by the community.
- After 3 years of hard work on software improvement, community input in ANISEED is now needed for the database content to be as good as its architecture.
- The curation tools we have developed are nice, logical and friendly to use, even if you are not very keen on computers...
- Community members who have entered a substantial number of experiments will be coauthors of the next ANISEED update paper.

We thank you for your attention and hope to soon welcome some of you among our curation team (please contact Delphine Dauga to create a ANISEED curation account at contact@aniseed.cnrs.fr).

[Ascidian News editor's comment: please specify if your data is for *Ciona intestinalis* or *C. robusta*!]

3. From **G rard Breton** (gerard-breton@orange.fr), Association Port Vivant, La Havre. **The 2014-2015 new observations on *Diplosoma listerianum* "balloons" in Le Havre harbor.**

In 2014, in the port of Le Havre, the divers of the Association Port Vivant noticed that some ascidians were curiously "ballooned" (see Ascidian news 74). The ascidian, first misidentified as *Didemnum vexillum*, proved, thanks to Fran oise Monniot, to be *Diplosoma listerianum*. During summer 2014, the "ballooned" *Diplosoma listerianum* were very abundant in the basins of the port of Le Havre. The phenomenon was not limited to the port of Le Havre, and to 2014! A picture by Thierry Derycke in April 2012 in the port of Le Havre shows a "balloon". Marcos Tatian reports "late August I participated in a campaign on board the vessel Puerto Deseado, along the Argentinean shelf. Surprisingly, the trawls carried a lot of these special formation or "balloons" of *Diplosoma*. They were very big and abundant." Jean-Louis Lenne gives a picture of such "balloons" of *Diplosoma listerianum* from a wreck, - 22 m, North Sea (14/07/2010) and another picture from the port of Boulogne-sur-mer, North Sea (18/10/2014). Fran ois-Xavier Huet photographed in the Rance Estuary on 31/10/2015 a typical balloon (Figure 1). Fran oise Monniot had observed the phenomenon long ago in the port of La Rochelle.

The question of the biological meaning of the balloons

In December 2014, Daniel Ingratta from his own pictures (Figure 4), then Gérard Breton from other divers' pictures (Figures 2, 3), remarked that the tunic of ballooned colonies develop groups of short, thin, thread-like expansions (ca 0.3 mm in diameter, several mm in length), better seen on a dark background. Some were attached to the substrate, other ones not. Gretchen Lambert wonders about the meaning of the “balloon” phenomenon and suggests that it could be an original mode of dissemination. The question and its answer are the same from Noa Shenkar and Marcos Tatian. Gretchen adds that the thread-like expansions could be linked to this supposed dispersal strategy, as reattachment structures. But this can be assessed only by an experiment that we tried in January 2015 and in October 2015.

January and October 2015 experiments

03/01/2015. Dive in the Bassin de la Barre (D.C. and P.A.). The number of *Diplosoma listerianum* – balloons has very much decreased. During one hour, two divers saw and collected only two balloons (1 and 1.3 cm in diameter), plus several which barely began forming vesicles. On the rock-filled side of the basin, *D. listerianum* has thoroughly disappeared.

On 03/01/2015 in the evening, I put in the bottom of a sea water tank with a bubbler some cleaned valves of mussel shells (concavity directed upwards). The two balloons collected by D.C., grey, deflated, were left each in a mussel valve in order to detect their ability to “re-fix” to a substrate. On 04/01/2015, one of the two balloons seemed to begin inflate again, the second one not. The tank was left at the exterior temperature of Le Havre, i.e. 7 to 10 ° C for six days. The two colonies of *D. listerianum* remained grey and deflated and, at the end of the six days, had not at all re-fixed to the support. Even a very soft movement of the mussel shell moved them in relation to the shell.

The scarcity of *D. listerianum* (balloons and ordinary ones), as observed by D.C. and P.A. in a basin in which this species (and its balloon form) was very abundant last fall suggests that its population was declining and in a wintry regression. The two collected balloon colonies were thus seemingly end-of-life colonies, of which the absence of re-fixing to the support is not significant. Thus, a new test was needed.

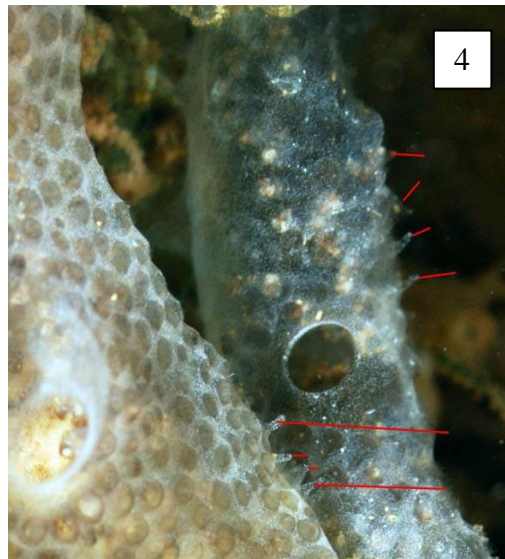
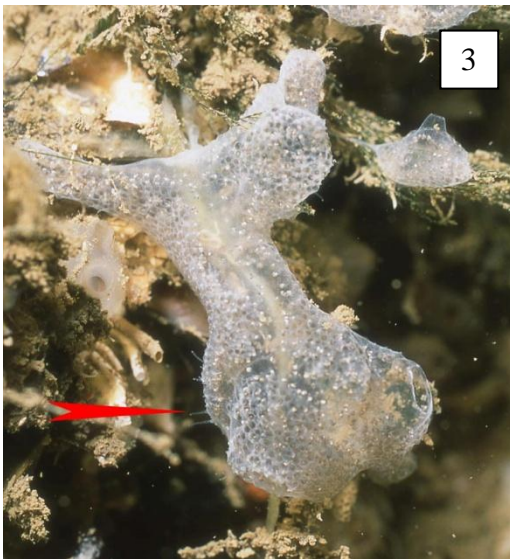
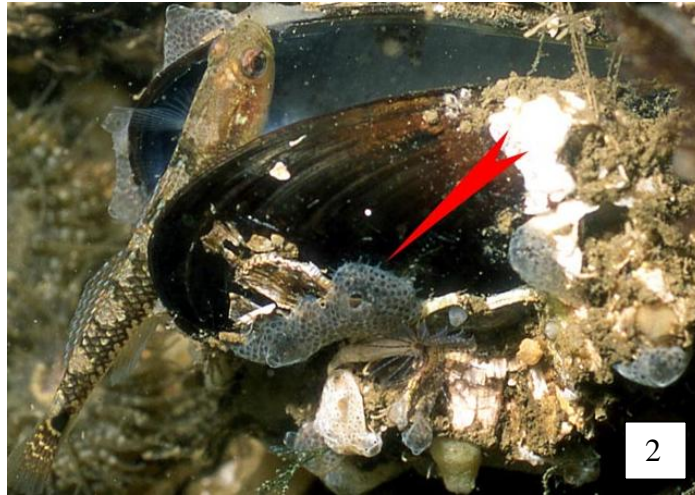
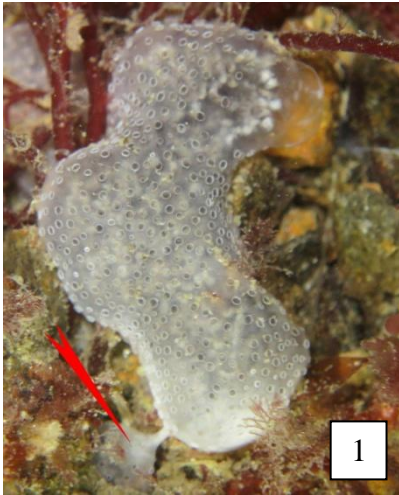
The same experiment was carried on by D.C. between 08 and 17/10/2015, with three balloons collected on 08/10/2015, with an exterior temperature between 10 and 12 °C. At the end of the experiment, the colonies were deflated and in very poor condition, and none was re-fixed to the substrate. The negative results of these trials is not conclusive: the biological signification of the “balloonization” of *Diplosoma listerianum* still remains hypothetical.

Figure 1: Rance Estuary (Brittany), 31/10/2015. © François-Xavier Huet. The arrow indicates a fixation to the substrate by an extension of the tunic.

Figure 2: Port of Le Havre, 07/12/2014. “Ordinary” *Diplosoma listerianum* (i.e. not ballooned), with thread-like extensions attached to a mussel shell. The fish is *Gobiusculus flavescens*. © Paul Leroy.

Figure 3: Port of Le Havre, 06/12/2014. © Marc Lacuisse. The arrow indicates the thread-like extensions of the tunic.

Figure 4: Port of Le Havre, 06/12/2014. © Daniel Ingratta. Enlargement of a close-up picture. The red lines indicate the thread-like extensions of the tunic.



Gérard writes further that *Perophora japonica*, now known from a number of locations in the Atlantic and Pacific, is expanding its population in the port of Le Havre.

THESIS ABSTRACTS

Examining genetic diversity and fusion abilities of an invasive colonial ascidian.

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Chapter 1: Invasive species cause both ecological and economical problems in their new habitats, either by directly harming or outcompeting native organisms. One such marine invasive species, *D. vexillum*, has recently been found globally and is spreading and altering habitats at an alarming rate. Global diversity of this colonial ascidian has previously been assessed using the barcoding mitochondrial gene, cytochrome c oxidase subunit I (COI), to determine that the native region includes Japan and that four other regions (western North America, eastern North America, Europe and New Zealand) are significantly lower in diversity. However, there have been few comparisons of diversity levels among populations

within a region or uses of higher resolution markers for the species. This study provides such an evaluation with 11 microsatellite markers in addition to COI to determine population structure and diversity of four locations within the western North American region. These locations have differing histories in terms of geographic distances to each other and other locations with *D. vexillum* within the region, boat traffic and aquaculture use. Analyses of the markers used in this study reveal surprising levels of differentiation and suggest that diversity and genetic isolation are affected by more than international shipping and proximity to such ports, and that regional boat traffic and gear movement can be important vectors of *D. vexillum* for both introductions and secondary spread to various points around the world.

Chapter 2: Fusion is a behavior often found in colonial organisms when two conspecific colonies come in contact with one another. However, it presents a puzzling conflict to fused colonies that bear the cost of contributing to shared structures if they are contributing fewer genes to the next generation. Kin selection has been proposed as a possible solution, but it is not evident in all ascidian species. In some species, like the well-studied basal chordate *Botryllus schlosseri*, fusion is dependent on matching alleles at particular loci, limiting fusion to close relatives, while in other species, like *Diplosoma listerianum*, there is no specificity when colonies fuse. A previous study inferred that *Didemnum vexillum* may also have genetic specificity for fusion, based on a negative correlation between fusion rate and measured gene diversity. The current study further investigates the specificity of *D. vexillum* by testing whether fusion behavior increases with increased genetic relatedness at three sites within the western North America region. Using microsatellite markers, high-resolution multi-locus genotypes of paired fusion assays were assessed. Relatedness values of colony pairs that fused were compared to relatedness values of colony pairs that did not fuse. There is a trend of higher genetic similarity correlating to higher likelihood of fusion. Weak correlation may be due to the use of neutral loci rather than a specific fusion gene, or to a decreased cost associated with fusion compared to *Botryllus schlosseri*. *D. vexillum* fusion involves tissue sharing, rather than vascular system integration found in botryllid ascidians. In *Botryllus schlosseri*, high specificity of fusion is limited to close relatives in theory to reduce the possibility of cell parasitism that occurs when the colonies' vascular systems fuse. This study provides further evidence that *D. vexillum* may also restrict fusion to genetically similar colonies in order to mitigate the inferred costs of fusing with unrelated colonies.

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