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Zoologie. — "On the spinispirae of Spirastrella bistellata (O. S.) Ldfd." By Dr. G. C. J. VOSMAER, Professor at the University of Leiden.

(Communicated in the meeting of January 30, 1909).

Some years ago (1902) I drew attention to the fact that there is confusion with regard to the terminology of certain sponge-spicules, and tried to clear this up. I arrived then at the conclusion that the spicules, which are generally called "spirasters", far from being a sort of "asters", i.e. polyaxon spicula, ought really to be considered as monaxons, the axis of which is a helix screw. I proposed for that kind of monaxons the term spiraxon (l.c. p. 105 and 112). In order to avoid further confusion I called the spined forms: spinispirae. I had some doubts about the supposition of some authors, that transitions between true asters and spinispirae really existed, because I never found them and failed to find any proof in literature (l.c. p. 105). On the one hand authors make a certain distinction between true asters (enasters) and "spirasters", but on the other hand consider both forms as belonging to the same group. Thus TOPSENT (1900 p. 21) distinguishes the genera Hymedesmia and Spirastrella on account of the fact, that the microscleres of the former genus are "euasters", of the latter "spirasters". It is generally accepted that the microscleres of Hymedesmia stellata are existence; but with regard to H. bistellata there is diversity of opinion and confusion. I believe this to be due to an erroneous conception of the spicules under consideration. Although I was convinced for myself, that these spicules were by no means (polyaxon) asters, but (monaxon) spinispirae, I have tried nevertheless to produce proofs for my statement by carefully studying the spicules treated in various ways. More especially I was led to do this in order to settle the question between LENDENFELD and TOPSENT about the sponge, which OSCAR SCHMIDT first described under the name of Tethya bistellata. Is it, as LENDENFELD suggests, a species of Spirastrella, or, as TOPSENT believes, one of Hymedesmia? Of course it is no Tethya; so far everybody agrees.

SCHMIDT (1862 p. 45) described a sponge, which he called *Tethya* bistellata, a name which he altered himself into Suberites bistellatus (1864 p. 36). Now LENDENFELD believed to have traced the sponge in his collection from Lesina and called it Spirastrella bistellata (1897 p. 55). From this TOPSENT dissented in 1898, alleging that

Tethya bistellata O. S. must be transferred to Hymedesmia, and consequently he called it Hymedesmia bistellata (1900 p. 125)¹).

Now I possess in my collection from Naples a sponge, which is beyond reasonable doubt SCHMIDT'S *Tethya bistellata*. I can affirm this especially because the spicules *absolutely agree* with those of a preparation I made at the time in Graz and which is labelled "Suberites bistellatus O. S. Origin. Schmidt." We may suppose, therefore, that LENDENFELD, TOPSENT and myself really examined the same sort of spicules, albeit that I must acknowledge that there is no *absolute* proof.

TOPSENT says, that the microsclera under consideration are euasters; he writes (1900 p. 123) that they are "sphèrasters de forme particulière... Chacune d'elles résulte de la congrescence latérale de deux sphèrasters à actines nombreuses, coniques, pointues et lisses." And later (p. 127): "les sphèrasters sont doubles. O. SCHMIDT a insisté sur ce caractère important, auquel l'espèce doit son nom." The question arises whether SCHMIDT's statement is of great value. In 1862 he said (p. 45) that some are "ganz eigenthumliche Zwillingsgestalten"; and further: "es sind also Doppelfiguren, welche einige Aehnlichkeit mit den Euastern haben." It must, however, not be forgotten that SCHMIDT at that time was unconcious of the sort of spicula which he called later (1868 p. 17) "Spiralsterne" or "Walzensterne" of which he mentions as characteristic "dass ihre Strahlen nicht Radien eines Centrum sind, sondern in Spiralstellung sich folgen."

According to LENDENFELD (1897) are the spicules under consideration "spirasters" and he gives some illustrations (l. c. Pl. VI fig. 59) which clearly show his conception of the thing. Both from his illustrations and from his description it follows that the axis is sometimes longer, sometimes shorter. LENDENFELD does not believe that "euasters" occur and suggests that SCHMIDT was perhaps misled by an optical illusion. If spicules are examined "deren Axen im Praeparat aufrecht stehen und daher verkürzt gesehen werden" they simulate euasters. i per

In spite of the fact that TOPSENT himself remarks, that in minute microsclera it is much more obvious that the centre is a line and not a point, this author does not consider them as spirasters but as double euasters. "Plus elles grandissent, plus la tige d'union se raccourcit. Sur les plus grosses, les deux centrums sont directement

¹) Actually the course of events was this: TOPSENT (1900 p 113) writes with regard to *Tethya bistellata* 0.5.: "je l'ai mise à sa place naturelle en 1892." However, in that article nothing is mentioned but the name *Hymedesmia bistellata* without reference to *Tethya bistellata*.

accolés..." Of course TOPSENT has not overlooked that spicula have a different appearance whether seen "de profil" or "de face"; but evidently he did not pay attention to intermediate positions such as can be seen if one allows them to turn over. I drew attention (1902 p. 170) to the fact that in almost all cases the twisted character becomes plain enough by applying the above device.

However, there are some more methods to make out the shape and the structure of spicules (Cf. VOSMAER & WIJSMAN, 1905 p. 745).

One of these methods is heating. In using this method it is, however, not indifferent in what way it is applied. If isolated spicules (e. g. styli of *Tethya*) simply dried in the air, are heated on a platina spatula immediately above the flame, a brownish colour soon becomes visible. If they are further heated the brownish tinge turns into white. It then frequently occurs that a crackling noise is heard and that spicules or portions of spicules jump off from the spatula. Such spicules, seen under the microscope, generally appear to be cracked or broken; they are brown or black, some were quite misshapen as if the spicopal had been partly melted.

How can these phenoma be explained? BOWERBANK ascribed the brown or black colour to carbonised organic matter, but Kölliker proved that the colour can certainly not wholly be explained in this way. Indeed, in some parts the colour is brown only in transmitted light, whereas it is white in reflected light; consequently KÖLLIKER declared those parts to contain microscopical air-bubbles. Quite correctly BÜTSCHLI (1901 p. 240) remarks that Kölliker where he speaks of "Luft", in fact means "Gas". WIJSMAN and myself have demonstrated (1905 p. 28), that spicopal is a form of hydrated siliceous acid, which can give off water in an atmosphere dried by P_aO_b . It is, therefore, very likely that when the spicules are treated as described above, a portion of the water becomes water-vapour. The tension of the heated globules of steam of course can be great enough to make the spicule explode. This explains at the same time the crackling noise and the jumping off from the spatula. Still, it need not come to this; hence we see some spicules only slightly cracked, not broken or deformed.

If, however, spicules are not simply dried in the air, but, by slowly warming on asbestus for several days or by P_sO_s , water is taken from them, and they are afterwards very carefully heated, then it is possible to prevent any cracking. Spicules treated in this way show quite other details. First of all the carbonised central thread is clearly visible. In some spicules the rest of the spiculum remained quite transparent; in others a brownish colour is to be seen

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on special places. Seen with reflected light these places are white; on the whole those places have a granular or frothy appearance.¹) As a rule the lamellar structure is very conspicuous. In certain cases the carbonised spiculum sheath is likewise visible. It also happens that the carbonised and shrunken central thread is seen as a flexuous, continuous or broken, black string lying within the central canal (fig. 20—21). Of course the best microscopical figures are obtained if the spicules are examined in a medium the index of refraction of which is equal to or comes very near that of the spicopal.

Controlling experiments sufficiently prove that no artefacts or anything of that sort come into play through which no conclusion can be drawn about the structure of the spicule. The spicopal being in slowly by dissolved the method published by WIJSMAN and myself, the microscope reveals facts which are in perfect accordance with those obtained in the way described above. One may also combine the two methods - heating and dissolving; again the results are the same if one follows the process under the microscope. Suppose one observes in heated spicules a black central thread with a brownish surrounding; suppose the object is mounted in glycerine of about the same index of refraction as the spicopal, the external limit is clearly visible as a delicate dark line (fig. 22). Some time after the action of the hydrofluoric acid the spicule appears as drawn in fig. 23. The silica begins to be dissolved as soon as the hydrofluoric acid has penetrated the spiculum sheath; the external delicate line remains visible but at some distance the limit of the spicopal, now thinner, becomes visible. The distance between the sheath and the limit of spicopal becomes gradually larger, the brownish surrounding of the central thread disappears and finally nothing is left but the carbonised central thread and the likewise carbonised sheath (fig. 24). This proves, that the brownish colour arround the axial thread does not originate from carbonised organic matter.

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¹) Bürschli admits as is well known, that in spicules which are not heated likewise little holes occur and that these holes simply become larger by the process of heating and consequently better visible. He says (l.c p. 248): "Das Auftreten der feinwabigen Struktur beruht darauf, dass eine solche auch schon in der nicht geglühten Nadel besteht, jedoch zu fein, um mikroskopisch sichtbar zu sein. Beim Glühen tritt eine Verdampfung des in den Wabenhohlräumchen eingeschlossenen Wassers ein und damit eine Erweiterung derselben bis zur Sichtbarkeit. Für diese Ansicht spricht vor Allem die Beobachtung, dass wenigstens in einem Fall auch eine nicht geglühte Nadel.... den wabigen Bau der Schichten deutlich zeigte." Apart from the question whether in unheated spicules a frothy structure really occurs or not, it is certain that the dark colour of heated spicules is due to little holes, void of air or filled with some gas, say water-vapour.

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The experiment can be modified in the following way. Isolated spicula are brought into acid fuchsine; if the hydrofluoric acid is now allowed to act on the spicules the spicopal will be dissolved, whereas the sheath and the central thread will be stained red. In both cases the silica is dissolved; in the former case the thread and the sheath are visible because they are black (carbonised), in the latter case because they are red. In the original experiment the spicopal is only optically dissolved.

What has been said for the styli of *Tethya* holds true m.m. for other spicules of Demoterellida. The structure of several spicules - monaxons, tetraxons or polyaxons - is fundamentally the same; in details there are important differences. However, I do not wish to speak about them in this paper. I have only mentioned as much as seemed to be necessary to show that by the described methods we are able to demonstrate most plainly the central thread. This can be done also in those cases in which the thread is not visible under ordinary circumstances, e.g. if the spicules are very minute or irregularities of the surface prevent it. Thus, for instance, in Tethya no central threads are visible in the oxyasters or at any rate they are not present beyond doubt¹). If these spicules are heated with great precaution they look under the microscope like fig. 19. It depends, as in other spicules, on the grade on heating whether the thread will be blackened only or with it its surroundings. Independently of this it is evident that the axes originate from one point.

Applying the heating method to the spicules in question of Spirastrella bistellata (O. S.) Ldfd. the microscope reveals pictures as drawn in fig. 1-3. It is most evident that we have here an axis exactly like that which unquestionable spinispirae possess. Such images are entirely unexplainable if the spicules are considered as congrescences of two euasters. They fully exhibit their true nature of spicules belonging to my group of *a*-spiraxons (1902 p. 112). Although I suppose this to be convincing, I applied moreover the dissolving method. It seems rather a paradox that the shape and the structure of a siliceous spicule can be cleared up by dissolving the silica. Still it is a fact, as I have frequently learned. WIISMAN and myself (1905 p. 18) confirmed BÜTSCHLI'S observations of 1901, that the dissolution of spicopal may proceed in more than one way. Only we have given another explanation of the fact. According to our

¹) On the whole spongiologists speak about the central thread as a constant feature of spicules. As a matter of fact stands that the presence of a thread is proved only in some cases and that in numerous microscleres nobody saw it. As far as I know only KOLLIKER found it in oxyasters of Tethya (1864, Pl. 1X, fig. 2).

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conception the spicopal which limits the central canal is more easily dissolved than that of subsequent layers. It seems that this is likewise the case for the radii of oxyasters of *Tethya*. It is probable that this depends on a difference in the quantity of water the "gel" contains. Now we observed that in pointed undamaged spicules, where consequently the central canal is shut, the funnelshaped dissolution is not seen, at any rate not at the very beginning of the process. The apex simply becomes thinner and thinner till the dissolving agent reaches the neighbourhood of the central canal, in which case the "funnel" often appears. Consequently we have herein another method to prove the existence of a central canal.

On the other hand we may conclude from this, that, if a funnel never appears there is no central canal resp. no special layer of spicopal in the centre. Thus, for example, in spicules with spines, the latter disappear gradually and the spicule becomes gradually thinner. I have observed this phenomenon very distinctly in acanthostyli of an *Ectyon* from Naples.

If the latter method is now applied on *Spirastrella bistellata* (O.S.) Ldfd. we see, that the pointed processes of the spinispirae become thinner and shorter, and finally disappear whereas the rest of the spiculum later becomes thinner (fig. 4—18). The microscopical images one sees during this process leave no doubt with regard to their structure. The more the spines dissolve, the more it becomes evident that we have to do with spinispirae.

Moreover, it follows from the above experiments that the spines of these spinispirae are of quite another nature than the actines of the *Tethya*-asters. In the former case (*Spirastrella*) we have to do with local extuberances of spicopal destitute of any central thread or canal. In the latter case (*Tethya*) we have organic axes. Indeed, the former spicules are monaxons, the latter are polyaxons.

Consequently the microsclera of Spirastrella bistellata (O.S.) Ldfd. are indeed spinispirae. Since LENDENFELD, TOPSENT and myself believe to have found sponges, which are identical with Tethya bistellata of OSCAR SCHMIDT, the species belongs as little to Hymedesmia as to Tethya. For the moment there is not sufficient evidence not to bring it to Spirastrella. The name for Tethya bistellata O.S. has to be, therefore, Spirastrella bistellata (O.S.) Ldfd. I believe with TOPSENT that it is identical with Spirastrella cunctatrix O.S.

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EXPLANATION OF THE PLATE.

(Fig. 1-21 are drawn 500 times magnified; fig. 22-24 still more magnified).

- Fig. 1-3 Spirastrella bistellata; spinispirae carefully heated. In 3a only the central thread (carbonised) is drawn, lying in the central canal.
- Fig. 4—18 Id. Influence of hydrofluoric acid. In figs. 4, 5 and 6 the acid has acted for a short time; only the spines begin to be dissolved. In fig. 7—12 the process is advanced; the "axis" becomes more and more obvious. In fig. 13—16 this is still more the case.
- Fig. 19 Tethya lyncurium; oxyaster after carefully heating; distinct, (carbonised) central thread.
- Fig. 20 ld. Middle piece of a stylus, carefully heated. Black (carbonised) central thread, entirely filling up the central canal.
- Fig 21. Id. Id. Shrunken, bent and broken central thread in the somewhat brownish central canal.
- Fig. 22-24 Id. Id. Slightly more heated and brought into the hydrofluoric camera of VOSMAER & WIJSMAN; α . central thread. b. brownish layer around the central canal, s. brownish spiculum sheath. In fig. 22 it is seen at the beginning of the experiment; the sheath lies immediately on the external spicopal; the little granula are adhering particles, not belonging to the spiculum. In fig. 23 the hydrofluoric acid has penetrated the sheath and dissolved the peripheral layers of spicopal, the limits of which are marked c; the sheath remained in its place. In fig. 24 all spicopal is dissolved; only the carbonised sheath s lies as a very delicate cylinder around the central thread.

Leiden, 2 Jan. 1909.



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