

Gastrochaena und Clavagella zu Aspergillum. Ebenso wenig erscheint danach Dall's<sup>7</sup> Eintheilung der Bivalven in die drei Ordnungen: Anomalodesmacea, Prionodesmacea und Teleodesmacea, als den verwandtschaftlichen Beziehungen der in diesen Ordnungen vereinten Formen entsprechend. So umfaßt die Ordnung der Anomalodesmacea folgende Unterordnungen: Solenomyacea, Anatinacea, Myacea, Ensiphonacea, Adesmacea; die Ordnung der Prionodesmacea die: Nuculacea, Arcacea, Naiadacea, Trigoniacea, Mytilacea, Pectinacea, Anomiacea, Ostreacea; die Ordnung der Teleodesmacea die: Tellinacea, Solenacea, Mactracea, Carditacea, Cardiacca, Chamacea, Tridacnacea, Leptonacea?, Lucinacea, Isocardiacca?, Veneracea.

Wien, Juli 1892.

## 2. A Method for Making Paraffine Sections from Preparations stained with Ehrlich's Methylenblue.

By G. H. Parker, Cambridge, Mass., U. S. A.

eingeg. 27. Juli 1892.

In the course of some studies on the ganglion in the optic stalk of the crayfish, the writer found it necessary to devise a method for making paraffine sections from preparations in which the nervous elements had been stained with Ehrlich's methylenblue. The following preliminary account, prepared at the suggestion of Herr Geheimrath F. E. Schulze, gives the essential steps in the method which was finally adopted.

In order to stain the elements in the nervous system of a crayfish,  $\frac{1}{10}$  to  $\frac{1}{20}$  ccm of a 0,2 % aqueous solution of methylenblue was injected into the ventral blood sinus, the animal afterwards being kept alive in a glass aquarium.

In about 15 hours many of the ganglion cells and nerve fibres in the peripheral as well as the central nervous organs were stained intensely blue.

Preparations made in this way, after being removed from the animal, retain their color only about an hour, but as is well known they can be made more nearly permanent by treating them with reagents which precipitate the methylenblue, such as picric acid, ammonium picrate, potassic iodide, potassic ferro-cyanide, chromic acid or corrosive sublimate. Of these reagents the one last named, in addition to being an excellent fixing reagent, yielded the most satisfactory preci-

<sup>7</sup> Dall, On the Hinge of Pelecypods and its Development, with an attempt toward a better subdivision of the group. American Journ. of Science. vol.38. 1889. p. 460.

pitae; in a well stained ganglion or nerve a cold, concentrated, aqueous solution of corrosive sublimate converts the methylenblue into a finely grained purplish precipitate.

In order to bring such a preparation into paraffine, it must first be dehydrated. The dehydration cannot be accomplished by the use of alcohol, for this fluid dissolves the precipitated color. As a substitute for alcohol two fluids, acetone and methylal, were tried. In acetone the precipitate is as soluble as in alcohol and in pure methylal it is also slightly soluble, but in methylal containing some corrosive sublimate it remains unaffected. The tissue was therefore dehydrated in a solution composed of 1 gramme of corrosive sublimate and 5 ccm of methylal.

The preparation, after being dehydrated, is of course permeated with a strong solution of corrosive sublimate in methylal. To free it from corrosive sublimate and to replace its methylal gradually with xylol is the next step. This is in part accomplished by putting it next into a mixture composed of two parts xylol, one part pure methylal and one part of the dehydrating mixture of methylal and corrosive sublimate. In this mixture some of the corrosive sublimate is washed out and a part of the methylal is replaced by xylol. After remaining in this mixture a short time the preparation is next placed in a considerable quantity of xylol. Here it should remain till all the methylal is replaced by xylol and the corrosive sublimate is completely washed out. As the last named substance is only slightly soluble in xylol, the preparation should stay in this fluid some four or five days. At the end of this time it may be either mounted in xylol balsam and studied as a transparent object or imbedded in paraffine and cut in the usual manner. The sections should be fixed to the slide with Schällibaum's collodion and not with Mayer's Albumen which discharges the color. Whole preparations or sections made in this way are serviceable for study for several weeks, but after an interval of a month the finer details in them are likely to fade.

The principal difficulties met with in employing this method are three: a semicrystalline condition of the precipitate due apparently to overaction of the corrosive sublimate; incomplete dehydration and imperfect removal of the corrosive sublimate. Remedies for these troubles easily suggest themselves.

The essential steps in the method can be recapitulated as follows, the lengths of time given being those required for a satisfactory preparation of a ganglion in the ventral nerve chain of the crayfish.

- 1) Cold, saturated, aqueous solution of corrosive sublimate for 10 minutes.

2) Solution A: methylal 5 ccm + corrosive sublimate 1 g, for 15 minutes.

3) Solution B: methylal 1 vol., solution A 1 vol., xylol 2 vols., for 10 minutes.

4) Pure xylol in considerable quantities for 4 or 5 days.

5) Mount preparation in xylol-balsam or imbed in paraffine and cut sections, which should be fixed to the slide with Schällibaum's mixture.

In conclusion I wish to express my thanks to Herr Geheimrath Schulze in whose laboratory in Berlin the work was done, and to his assistants Dr. Korschelt and Dr. von Mährenthal for the many kindnesses they have shown me in the course of my work.

### 3. Neue Diplopoden der paläarktischen Region.

Von C. Verhoeff, Bonn a./Rh.

eingeg. 31. Juli 1892.

Die nachfolgenden Auseinandersetzungen der mir vorliegenden neuen Arten genügen um dieselben wieder zu erkennen. Ich beabsichtige jedoch denselben später weitere Details hinzuzufügen, vor Allem sollen auch die Begattungsorgane der Männchen, welche mir meistens bekannt sind, eine genaue bildliche Darstellung erfahren.

Diese Thiere stammen zumeist aus der portugiesischen Fauna und ich kann jetzt bereits hervorheben, daß die Diplopoden jenes abseits gelegenen Theiles der paläarktischen Region sich zum Theil durch auffallend ursprüngliche Merkmale auszeichnen.

Die neu beschriebene *Glomeris* ist recht interessant durch ihre merkwürdige Fleckenstellung (cf. unten).

#### 1. *Julus alemannicus* m. ♂ ♀<sup>1</sup>.

♀ 18—23 mm lang, ♂ 22—23 mm lang. Breite 1,2—1,5 mm. 49—52 Segmente, 85—93 Beinpaare, 2—4 beinlose Endsegmente.

♀ aus Kopf und 49—52 Segmenten bestehend; ♂ aus Kopf und 50 Segmenten bestehend.

♀ mit 93 Beinpaaren hat bei 52 Segmenten drei beinlose Endsegmente.

♀ mit 85 Beinpaaren hat bei 49 Segmenten vier beinlose Endsegmente.

♂ mit 89 Beinpaaren hat bei 50 Segmenten zwei beinlose Endsegmente.

<sup>1</sup> Vorkommen: Neckarthal bei Heidelberg. Neuerdings fand ich ihn zahlreich in der Schweiz, worüber demnächst berichtet wird.

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