THE METAMERISM OF NEPHELIS:
WITH A DESCRIPTION OF
NEPHELIS LATERALIS (VERRILL);
A DISSERTATION, VOL. 15, NO. 1,
PP. 17-72

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CHARLES LAWRENCE BRISTOL

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A DISSERTATION

Submitted to the Faculties of the Graduate Schools of Arts, Literature, and Science, in candidacy for the degree of

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BA

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THE METAMERISM OF NEPHELIS.

A CONTRIBUTION TO THE MORPHOLOGY OF THE NER-VOUS SYSTEM, WITH A DESCRIPTION OF NEPHELIS LATERALIS.

CHARLES L. BRISTOL.

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INTRODUCTION.

THE work which forms the basis of the present paper was begun in 1891 at Clark University, Worcester, and continued at the Marine Biological Laboratory at Woods Holl and Chicago University, and I wish to acknowledge here my indebtedness to the authorities of these institutions for the facilities and the Fellowship privileges granted to me. To Professor Whitman, at whose suggestion I began the investigation, I am deeply indebted for aid and encouragement and for many courtesies extended to me. I am under obligations to Dr. Wm. M. Wheeler

for aid and advice, and to Prof. S. A. Forbes, of the University of Illinois, for the privilege of examining the specimens of Nephelis collected by him under the auspices of the U. S. Fish Commission in the Yellowstone region in Wyoming.

HISTORICAL.

In 1767 Linné enumerated nine species of leeches in one genus, Hirudo. This classification was followed by most later authors, for example, Cuvier, Blumenbach, Carena, and Dumeril, until about 1817, when Savigny, in his Système des Annelides, announced the separation of Linné's genus into seven genera. The name Nephelis appears in this work for the first time, although Oken set this leech apart from Hirudo in 1815 under the name Helluo, which genus was to include all fresh-water leeches not provided with jaws. In 1818 Lamarck, at the suggestion of Blainville, proposed the name Erpobdella, which Blainville (1828) urged for acceptance because it contained the descriptive part "bdella." In 1826 Moquin-Tandon adopted Savigny's name Nephelis and continued it in the second edition of his Monographie des Hirudinées (1). The name has since become generally accepted, notwithstanding the fact that Oken's Helluo holds priority and Lamarck's Erpobdella is more descriptive.

The first description of Nephelis in America was made by Thomas Say (2) in 1824 under the name of *Hirudo lateralis*. In 1872 Verrill (3) changed this to *Nephelis lateralis*, which, for reasons given in another part of this paper, I have given to the leech I have studied.

METHODS.

The leeches are easily kept in aquaria, for which I used the low glass dishes known as crystallizing dishes, or white earthen-

A. Moquin-Tandon: Monographie de la famille des hirudinées. Paris. 1846.
 T. Say: Major Long's Second Expedition to the Source of St. Peter's River, vol. ii, Appendix to the Natural History. 1824. (Republished in Dissing's

Système Helminthologique, vol. 1.)

3 A. E. Verrill: "Synopsis of the North American Fresh-water Leeches." U. S. Fish Commissioner's Report for 1872-74. (Refers to the American Journal of Science, vol. iii, 1872.)

ware cooking dishes, known to the trade as nappies. In some instances I supplied the aquaria with a layer of mud and bottom dibris, together with a few plants such as Ceratophyllum or Valisneria. When such an aquarium is covered with a glass plate it will keep fresh and clean for a long time and will furnish considerable food for the leeches. Generally, however, I used the plain dish, cleaning out the débris and slime and changing the water when necessary. I fed chopped freshwater clams, but I do not doubt that salt-water clams will serve as well. I have kept individuals for over a year in normal condition and have raised many young under these conditions. When they are first transferred to the aquarium it must be covered for a day or two, to prevent escape. For superficial examination the leeches were killed in very dilute chromic acid, 1/6 to 1/3 per cent solutions. There is one period just before the acid penetrates very deeply when the surface markings stand out very clearly. The leeches usually extend themselves very well, and if killed in a wax tray they may be guided by pins. The best medium for histological details is a 1/4 to 1/2 per cent solution of chromic acid, allowed to act for at least 24 hours. The stains used were borax carmine, Delafield's haematoxylin, and Bizzozero's picro-carmine. The macroscopic characters of the nerve chain were studied from maceration preparations. The leech is killed in a 20 per cent solution of nitric acid and left in it for from 24 to 36 hours, or until the skin and muscles can be easily removed with a porcupine bristle or a glass rod drawn out to a point. These were all carefully dissected away, leaving the chain entire. thorough washing, the chain may be slightly stained in borax carmine and mounted in glycerine.

A number of details were worked out by the use of Haller's fluid. For example, the head was cut off, slit open on the ventral or dorsal side as wished, and killed in Haller's fluid while it was flattened under a piece of glass. After two days the specimen was transferred to glycerine.

The method that has given me the best results for nerves and sense organs is a gold chloride process kindly given me by Miss Julia B. Platt. It is so simple, so sure, and so exquisitely delicate in some of its effects that it deserves extended use. It may be used with equal success on vertebrate or invertebrate, adult or larval tissue. It must be adapted to the tissue studied; but this can easily be done after a few experiments. The formic acid appears to be the variable factor, and upon its strength and the time it acts depends the measure of success. I give the procedure applicable to Nephelis.

The leech is killed in a 10 or 15 per cent solution of formic acid, left from 5 to 10 minutes, and then put without washing into a 1 per cent solution of gold chloride for 25 minutes. From this it is transferred, without washing, into a large volume of 1 per cent formic acid, and left for 12 or 18 hours, or until reduction has taken place. It is next washed, passed through the alcohols to chloroform, and then imbedded in paraffin. The sections were cut 18 micra thick. The specimen will appear a rich purple when the reduction has taken place under the best conditions. The precautions are: to use small pieces of material, not thicker than 5 mm., to avoid maceration by reducing the strength of the formic acid and the time of action. My solutions were all well sunned, but no especial precautions were observed.

In tracing out the innervation of the somites it was necessary to examine long, continuous series of sections, and sometimes it was necessary to check results found in one somite by comparison with the next somite. The following method was used which would apply to other purposes. An ordinary library reference card, about 8 cm. by 10 cm., is ruled so as to include as many small rectangles in the same number of rows as the slide to be examined contains sections. The unused margin serves for making notes. An ordinary check mark denotes that the section occupying the same place on the slide that the rectangle does on the card has been examined but does not contain the element under examination. Initials, symbols, different colored pencils, etc., may be used to indicate various details, and each card is numbered the same as the slide. After a number of slides have been carefully plotted in this manner, the cards may be arranged in series and studied as a map. It furnishes an excellent reference-card system for any set of serial sections, and permits a rapid glance at the order of sequence of any character in different somites or individuals.

SYSTEMATIC.

Nephelis differs from nearly all other leeches in the external topography of the somite. While the somite in the Hirudinea, as a group, is characterized by prominent sense organs on the first ring, in Nephelis these are conspicuously absent, save on a few segments near the anus and in rare instances on a few rings near the mouth. The absence of these characters has compelled investigators to resort to other criteria for the determination of species, such as color markings, and the occurrence of four stripes of pigment on the dorsal side is sufficiently well marked to furnish a criterion of generic, if not of specific, value. In Europe the only well-established species is N. octoculata Bergmann. Blanchard (4) says: "Jusqu'a Savigny, la seule espèce admise sans contest était la N. octoculata Bergmann: Savigny a distingué plusieurs espèces basées exclusivement sur les differences de coloration : mais aucune de ces espèces nominale n'est representée et n'est surement reconnaisible. En outre de la N. atomaria, nous croyons pouvoir séparer de l'ancienne N. octoculata plusieurs autres formes spécifiques bien distinctes."

Moquin-Tandon in the first edition of his monograph accepts the description given by Carena for *N. atomaria* as a species, but in the second edition lowers it to the rank of a variety of *N. octoculata*.

The first mention of the genus in this country that I have found was made by Thomas Say (2) in 1824 under the name of *Hirudo lateralis*, and this was changed by Verrill (3) in 1872 to *N. lateralis*. Leidy (5) described a form (1870) under the name *N. marmorata*. Verrill describes four species of Nephelis found in the United States and says concerning three of them:

⁶ R. Blanchard: "Courtes notices sur les Hirudinées, III. Description de la Nephelis atomaria Carena." Bull. de la Soc. Zool. de France, tome xvil, p. 165, 1892.

[§] Jos. Leidy: "Description of Nephelis punctata." Proc. Acad. Nat. Sci. of Philadelphia, p. 89, 1870.

"When a larger series of living specimens from various localities can be studied, the three preceding forms (N. lateralis, N. quadristriata, N. marmorata), admitted here as distinct, may prove to be mere varieties of one species, no less variable than N. vulgaris of Europe." The fourth species, N. fervida, is described from specimens taken from Lake Superior and has eight ocelli. I have not collected a Nephelis answering to this description.

The genus is widely distributed in the United States. My own collections have been made in Massachusetts, Connecticut, Illinois, New York, and South Dakota. I have received specimens from Mr. A. J. Hunter, of Toronto, collected near Toronto, Can., and Professor Forbes, of the University of Illinois, kindly loaned me for examination the specimens of Nephelis collected by him in the Yellowstone region in 1890. Verrill records collections from Maine, Massachusetts, Connecticut, New Jersey, Wisconsin, Nebraska, Colorado (at an elevation of 9000 feet on Longs Peak), and from the waters of Lakes Superior and Huron. The area included covers about 35 degrees of longitude and 10 degrees of latitude; it embraces the Atlantic slope, the Great Lake Region, the Missouri Valley, and the Rocky Mountains.

Investigations on my own collections lead me to agree with Savigny, Moquin-Tandon, and Verrill that it is difficult to distinguish species by the criteria used by them, color and color markings, and to disagree with the methods and results, published by Lindenfeld and Pietruszynski (6), who rely on these features exclusively. My first attempts to classify the specimens which I collected were naturally based on the descriptions given by previous investigators, but it proved so difficult a task to determine what value to place on the various statements of color, and so many of my specimens could with equal propriety be placed in either of two or three categories, that it became evident that some different method of diagnosis would be necessary. The necessity of going beyond color markings was plainly shown by the following experiments.

⁶ Von Lindenfeld und Pletruszynski: "Beiträge zur Hirudineen fauna Polens." Reviewed by Nusbaum. Biol. Centralblatt, Bd. xii, p. 55, 1892.