

NUCELLICOLIDAE: A NEW FAMILY OF ENDOPARASITIC
COPEPODS (POECILOSTOMATOIDA) FROM THE DOG
WHELK *NUCELLA LAPILLUS* (GASTROPODA)

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ABSTRACT

The external morphology of the adults of both sexes and of the late male copepodid of *Nucellicola holmanae*, new genus, new species, parasitic in a gastropod mollusc, *Nucella lapillus*, is described. A new family within the order Poecilostomatoida is erected to accommodate this monotypic genus. The adult parasite is found within the viscera of the host and is presently recorded only on the North Sea coast of England.

The Copepoda contains well over 11,000 species (Humes, 1994), exhibiting an extremely wide diversity in form and mode of life. Copepods have colonized habitats encompassing a considerable range of salinity and temperature regimes, and an immense vertical range from ocean trenches to mountain lakes (Huys and Boxshall, 1991). Many copepods are plant and animal associates, parasitizing virtually every phylum of animals from sponges and cnidarians to vertebrates, including mammals. Many species are parasitic on fishes (Kabata, 1992) or are associated with marine invertebrates (Gotto, 1993). The associates of marine invertebrate hosts display an enormous spectrum of structural diversity, varying in part with the degree of intimacy of association with the host: the more intimate the association, the more profound the transformation of the parasite.

Many of those parasitic on invertebrates belong to the order Poecilostomatoida, a number of families of which contain species that are known to parasitize molluscs world-wide. Those belonging to several families, including the Mytilicolidae Bocquet and Stock, 1957, Chitonophilidae Avdeev and Sirenko, 1991, and Splanchnotrophidae Norman and Scott, 1906, are parasitic exclusively on molluscs. In Britain only seven poecilostomatoid species are known to infest gastropod molluscs. These are the anthessiids *Anthessius leptostylis* (Sars, 1916) and *A. teissieri* Bocquet and Stock, 1958, the rhynchomolgid *Doridicola agilis* Leydig, 1853 (see Humes and Boxshall, in press), the lichomolgid *Epimolgus trochi* (Canu, 1899), the splanchnotrophids *Splanchnotrophus gracilis* Hancock and

Norman, 1863, and *S. brevipes* Hancock and Norman, 1863, and the mytilicolid *Trochicola entericus* Dollfus, 1914.

This paper reports the first endoparasitic copepod from a prosobranch mollusc in British waters. It was found during a study of the parasites of the dog whelk *Nucella lapillus* (L.) on the North Sea coast at Robin Hood's Bay, North Yorkshire, England. The specimens represent a new family, genus, and species of the order Poecilostomatoida.

MATERIALS AND METHODS

The dog whelks were collected from boulders lying on the surface of a rocky platform at Robin Hood's Bay, North Yorkshire (British National Grid Reference NZ 955045). The dog-whelk shells were cracked open using a vice, and the soft parts removed using forceps. These were studied under a dissecting microscope and any copepod parasites removed. The parasites were examined live in sea water, or preserved in 70% alcohol and subsequently cleared in lactic acid for morphological study. The females were placed on slides and the adult males and copepodids removed using fine needles. All animals were studied using differential interference contrast microscopy and drawings were made with the aid of a camera lucida.

For histological studies of host-parasite relationships and of the anatomy of the copepod, parasitized dog whelks were fixed in Bouin's solution for 2-3 days. After dehydration, the operculum was removed and the dog whelk was embedded in paraffin. Serial sections were made at a thickness of 8 μ m, mounted, and stained with Mallory's or Masson's triple stain.

RESULTS

Nucellicolidae, new family

Diagnosis.—Podoplea, Poecilostomatoida. Adult female body transformed, vermiform, lacking traces of segmentation; comprising anterior cephalic region, neck, and genito-abdominal trunk. No appendages present.

Trunk containing paired oviducts opening via paired gonopores. Adult male smaller than female, located in membranous sac at posterior end of female. Male comprising head with paired lateral lobes and rectangular trunk. Trunk containing paired testes. Male with pair of well-developed, subchelate maxillipeds. Late male copepodid stage cyclopiform, with 5-segmented prosome and 3-segmented urosome. Cephalosome carrying paired antennules and antennae, lacking trace of mouth or postantennary appendages. Four pairs of biramous swimming legs present on pedigerous somites. Anal somite with caudal rami bearing 3 setae.

Type Genus.—*Nucellicola*, new genus.

Remarks.—A new family is proposed to accommodate the new genus *Nucellicola* because it cannot be placed in any existing family of copepods. It is difficult to assess the affinities of such highly transformed and secondarily reduced parasites. They typically lack appendages and their gross body morphology can be misleading, since there are, among the endoparasitic copepods, numerous examples of convergent evolution toward a vermiform body which lacks external segmentation. However, *Nucellicola* can be distinguished from other poecilostomatoid families containing species parasitic on gastropod molluscs by particular diagnostic characters, as follows:

Anthessiidae Humes, 1986—body cyclopiform; all appendages retained.

Chitonophilidae Avdeev and Sirenko, 1991 [only species of *Cucculinika* Jones and Marshall, 1986]—adult female highly transformed, body divided into embedded rootlet system and posterior trunk; trunk external to host; adult male retaining paired antennae, lacking maxillipeds.

Lichomolgidae Kossmann, 1877 [only species of *Epimolgus* Bocquet and Stock, 1956]—body cyclopiform; all appendages retained.

Mytilicolidae Bocquet and Stock, 1957—body elongate, retaining external segmentation, most cephalic appendages, and swimming legs.

Philoblennidae Izawa, 1976—body transformed by swelling and fusion of prosomal somites, urosome segmented in both sexes; cephalic appendages and

swimming legs 1 and 2 typically retained.

Rhynchomolgidae Humes and Stock, 1972 [only species of *Doridicola* Leydig, 1853]—body typically cyclopiform, all cephalic appendages retained.

Splanchnotrophidae Norman and Scott, 1906—adult female prosome transformed by fusion of somites and by development of prosomal processes; urosome typically differentiated and distinctly segmented; male body modified cyclopiform, retaining cephalic appendages and swimming legs 1 and 2.

The new family is characterized by the transformation/reduction of external segmentation of the adults of both sexes, not just of the females as in several of these other families. Only the Chitonophilidae has both sexes transformed; this family can be readily distinguished by the presence of a highly developed rootlet system in females and by the possession of paired antennae and a common median genital aperture in males (Avdeev and Sirenko, 1991). An additional character distinguishing the new family is the structure of the swimming legs in the copepodid stage. The elongate, 1-segmented rami are unusual and the distribution of the exopodal setae, along the distal and inner margins, is unique within the Poecilostomatoida.

The new family is placed in the order Poecilostomatoida on the basis of the structure of the antennae in the copepodid stage. The antennae consist of a coxobasis and 2-segmented endopod, as is typical of poecilostomatoids. The presence of two distal claws is also common in poecilostomatoids but extremely rare in siphonostomatoids or the small number of parasitic cyclopoids. The presence of well-developed maxillipeds in the adult male is also typical of poecilostomatoids, many of which exhibit sexual dimorphism in having well-developed maxillipeds in males, but having reduced or no maxillipeds in females.

Nucellicola, new genus

Description.—See diagnosis of family above.

Nucellicola holmanae, new species

Type Material.—Holotype adult female with adult male allotype attached, plus 2 paratype females each

with male attached, deposited in collections of The Natural History Museum, London: Registration Nos. 1995.664 (holotype), 1995.665 (allotype male), 1995.666–667 (paratypes). Five additional paratype females with males attached, in collection of senior author. All type material endoparasitic in *Nucella lapillus* collected from intertidal zone on rocky exposed beach at Robin Hood's Bay, North Yorkshire, England (British National Grid Reference NZ 955045) where *N. lapillus* is abundant.

Description.—Adult female highly transformed; body elongate, cylindrical, lacking external traces of segmentation (Fig. 1A); comprising swollen anterior cephalic region, neck region, and long genito-abdominal trunk; relative lengths of head, neck, and trunk. 1:3:13. Length of figured adult (Fig. 1A) 6.05 mm. Cephalic and trunk appendages entirely lacking. Anterior cephalic lobe possibly serving as anchor process, better developed in smaller specimens. Trunk largely filled with paired ovaries and highly convoluted paired oviducts. Mean length of adult trunk $4.43 \text{ mm} \pm 1.08 \text{ mm}$ (SD) from 10 specimens examined. Oviducts milky white in color due to developing eggs; terminating in paired gonopores at posterior margin of trunk. Paired cement glands present, linked by commissure below genital aperture. Adult male located within membranous sac, attached adjacent to paired genital apertures at posterior end of female (Fig. 1A).

Male body highly transformed, comprising lobate cephalic region and ovoid trunk (Fig. 1B, C), lacking external traces of segmentation. Cephalic region with paired lateral lobes and median lobe located anteriorly on ventral surface. Median lobe possibly derived from labrum. Paired maxillipeds located ventrally posterior to median cephalic lobe. No other cephalic appendages visible. Trunk ovoid, tapering posteriorly. Paired anteroventral lobes located anteriorly on trunk, possibly associated with paired gonopores. No trunk appendages present. Trunk containing paired testes and paired vasa deferentia. Mean body length $0.44 \text{ mm} \pm 0.17 \text{ mm}$ (SD), mean width $0.34 \text{ mm} \pm 0.10 \text{ mm}$ (SD) based on 11 specimens examined. Maxillipeds (Fig. 1D) subchelate, comprising robust proximal segment and curved distal claw, presumably representing endopod.

Late copepodid stage (male). Body cycloform (Fig. 2A); prosome 5-segmented,

comprising cephalosome and 4 free pedigerous somites, urosome 3-segmented, anal somite bearing pair of caudal rami. Caudal rami armed with outer, inner, and accessory terminal setae. Total body length from 180–250 μm based on 9 specimens examined (excluding caudal setae), maximum width 114 μm .

Antennule 4-segmented (Fig. 2B); armature as follows: I–1, II–5+1 aesthetasc, III–2+1 aesthetasc, IV–5+1 aesthetasc; aesthetasc on second segment bipartite, long; all setae naked. Antenna uniramous, 3-segmented (Fig. 2C), comprising coxobasis and 2 endopodal segments; coxobasis and first endopodal segment unarmed, distal segment terminating in 2 short apical claws. All other oral appendages including maxillipeds absent.

Swimming legs 1–4 biramous, each comprising coxa, basis, and two 1-segmented rami. Endopod armed with 2 setae on distal margin in legs 1–4. Exopod armed with 4 setae along distal and inner margins in legs 1 (Fig. 2F) and 4 (Fig. 2E), and with 5 setae along same margins in legs 2 (Fig. 2G) and 3 (Fig. 2D).

Etymology.—The generic name *Nucellicola* is derived from the generic name of the host, *Nucella*, plus *-icola* from the Latin meaning inhabiting. The trivial name honors Ceri Holman who first found this parasite.

Position in Host.—The body is typically located within the visceral hump of the dog whelk, attached to the inner surface of the body wall of the host toward the tip of the whorl. This position appears to be the final resting place of the female, but occasionally females can be found elsewhere in the host, such as the kidney, midway through their presumed migration. Each female parasite is contained within a membranous tube, possibly of host origin, which is full of eggs or developing nauplii and functions as an egg sac. This tube extends from the body of the female through the host viscera to an origin in the mantle wall near the shell opening. The tube is highly convoluted within the host tissue, and, in serial sections, eggs and nauplii at various stages of development can be seen to occupy up to 80% of the cross-sectional area of the visceral whorl of the whelk. The eggs closest

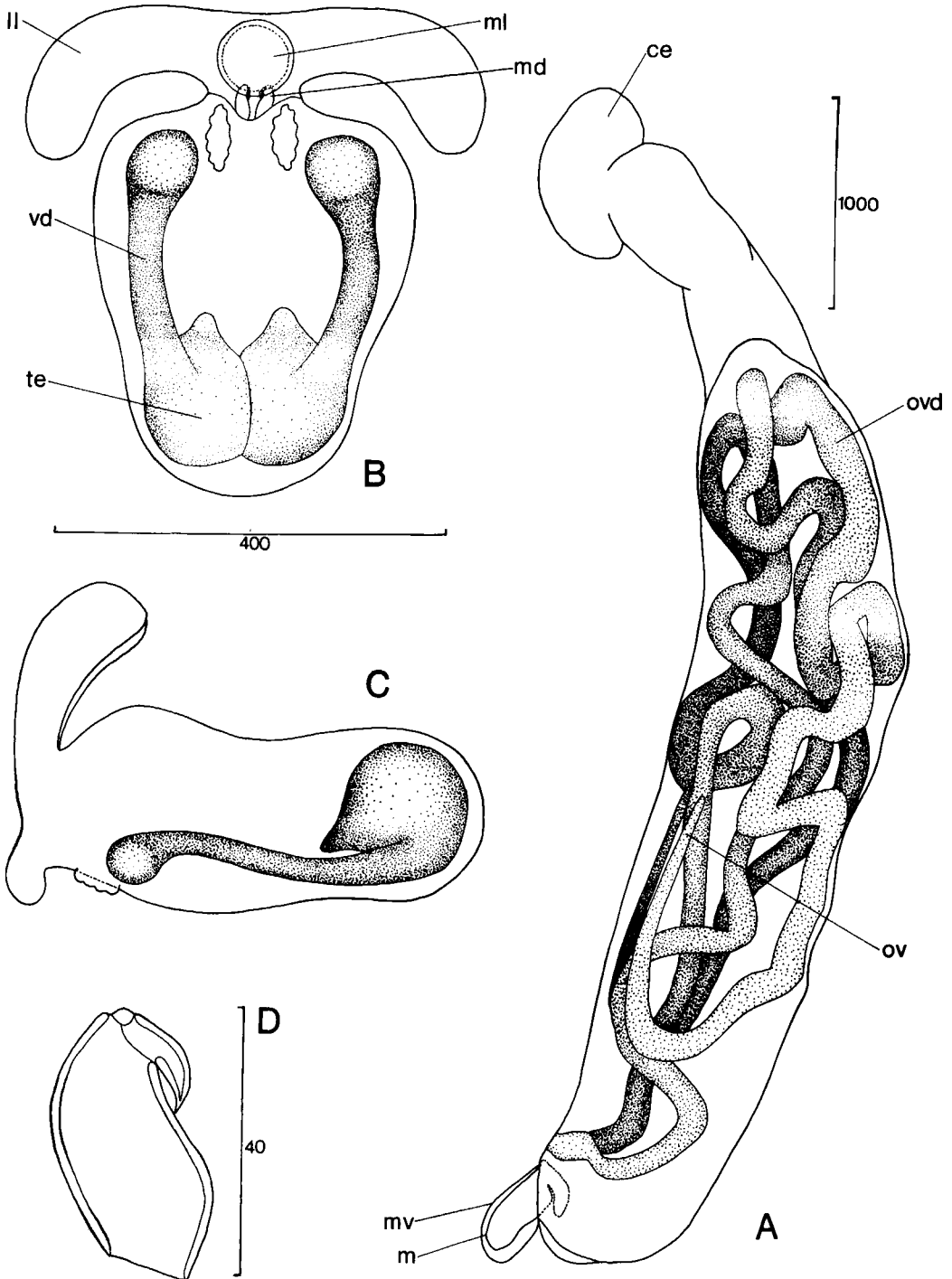


Fig. 1. *Nucellicola holmanae*, new genus, new species. A, adult female, lateral view; B, adult male, ventral view; C, adult male, lateral view, with dorsal surface uppermost; D, adult male maxilliped. Scale bars in μm . Abbreviations: ce = head, ll = lateral lobe, m = adult male inside membranous vesicle, md = maxilliped, ml = median lobe, mv = membranous male vesicle, ov = ovary, ovd = oviduct, te = testis, vd = vas deferens.

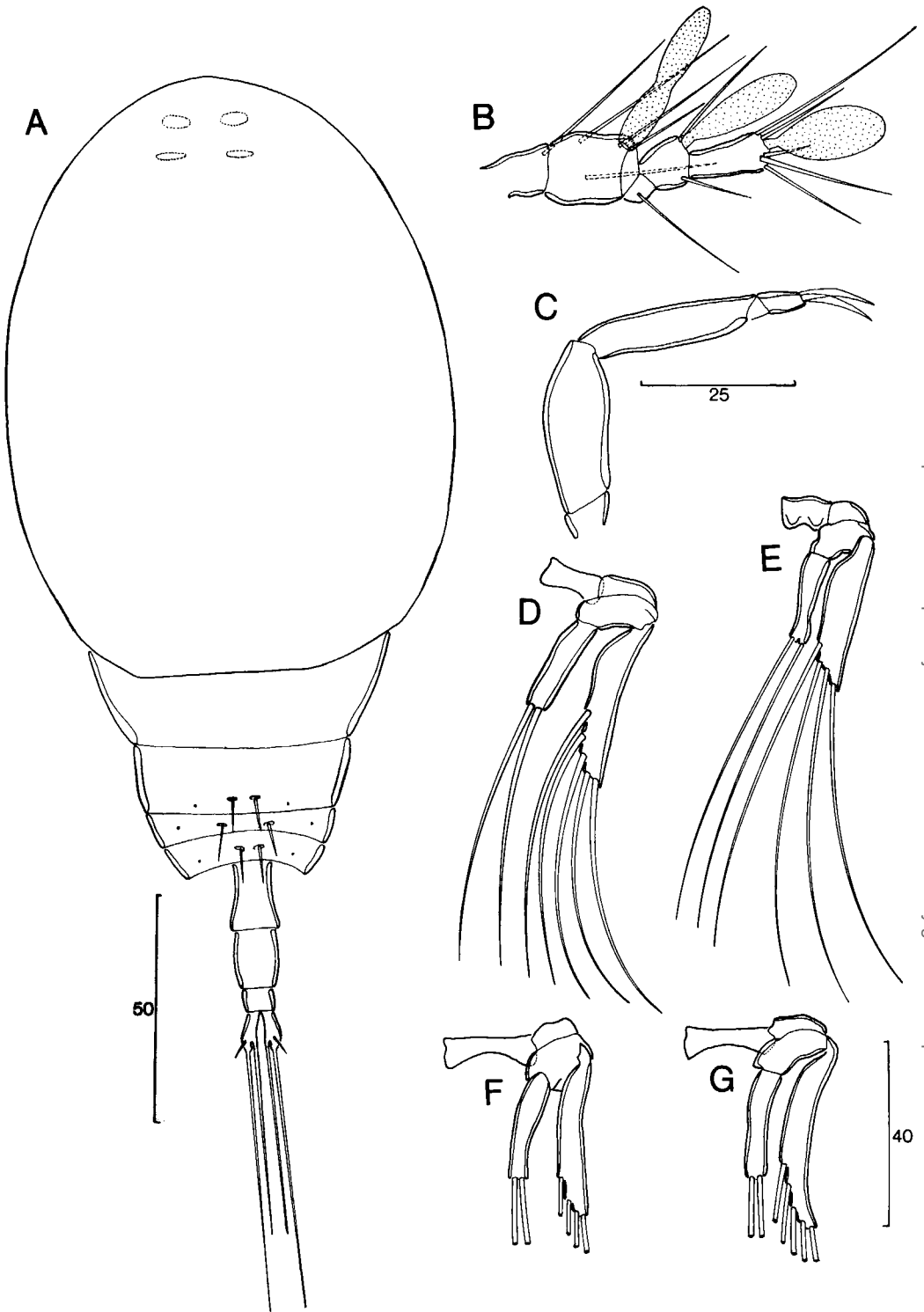


Fig. 2. *Nucellicola holmanae*, new genus, new species. Exuvia of late male copepodid (probably copepodid V). A, dorsal view; B, antennule; C, antenna; D, swimming leg 3; E, swimming leg 4; F, swimming leg 1; G, swimming leg 2. Scale bars in μm .

to the parasite are the least advanced in their development and thus, presumably, the most recently released. The nauplii farthest away from the female within the tube are the best developed.

Normally, each infected host contained only a single parasite, but, rarely, multiple infections of up to three mature females, each with their own egg tube, were found.

The male is found attached to the posterior end of the female and is contained within a saclike, membranous vesicle. It is positioned with its cephalic region close to the paired female genital apertures and its cephalic lobes are often partially embedded within a concavity in the posterior end of the female trunk. There is a single exuvia, probably of the male fifth copepodid stage, present with the male inside the male vesicle.

DISCUSSION

In every specimen examined the membranous vesicle at the posterior end of the female contains the adult male plus the exuvia of a copepodid stage. The copepodid is identified as an exuvia due to its complete lack of any internal organs or tissues. Therefore, it is probable that this copepodid is the final (fifth) male copepodid stage which, on finding a female, enters the membranous vesicle before molting into the highly transformed male adult.

It is remarkable that the late male copepodid stage lacks any trace of maxillipeds, because the adult male has a pair of fully developed, subchelate maxillipeds. These are probably used to attach to the female. In many other poecilostomatoids, for example, *Ergasilus sieboldi* (Nordmann, 1832), the development of the male maxillipeds is delayed so that the rudimentary maxilliped first appears at the copepodid V stage and is fully formed after the next molt to adult (Abdelhalim *et al.*, 1991). The absence of even a rudiment of the appendage at the penultimate stage, as in the new species, is unusual.

The mode of egg deposition of the new species is also unusual. Copepods that are parasitic in the tissues, rather than in the alimentary canal, of their hosts usually maintain an opening in the surface of the host and produce egg sacs externally. For example, in adult females of the family

Splanchnotrophidae the tip of the urosome protrudes out through the body wall of their nudibranch host, and bears paired egg sacs external to the host (Hancock and Norman, 1863). Members of the Xenocoelomatidae Bresciani and Lützen, 1966, such as *Aphanodomus* Wilson, 1924, similarly maintain an opening in the body surface of the host and produce paired external egg sacs (Bresciani and Lützen, 1974). Alternative strategies are exhibited by the bivalve-inhabiting genus *Pectenophilus* Nagasawa, Bresciani, and Lützen, 1988, which retains its eggs in a brood pouch within the body of the female (Nagasawa *et al.*, 1988), and by the nanaspidid *Allantogynus delamarei* Changeux, 1960, which lays its eggs in a large enveloping sac within the coelom of its holothurian host (Changeux, 1960). The system of egg production in *Nucellicola* exhibits a strategy similar to those of the two aforementioned species. Eggs are released into a membranous tube which extends from the parasite, located deep within the host, to the wall of the mantle at the shell opening. This represents a further step in the evolutionary trend exhibited by the siphonostomatoid *Pennella* (Oken, 1816), which creates an extensive invaginated pocket inside the body wall of the host. This pocket has an opening to the exterior from which the egg sacs protrude (Kabata, 1979).

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