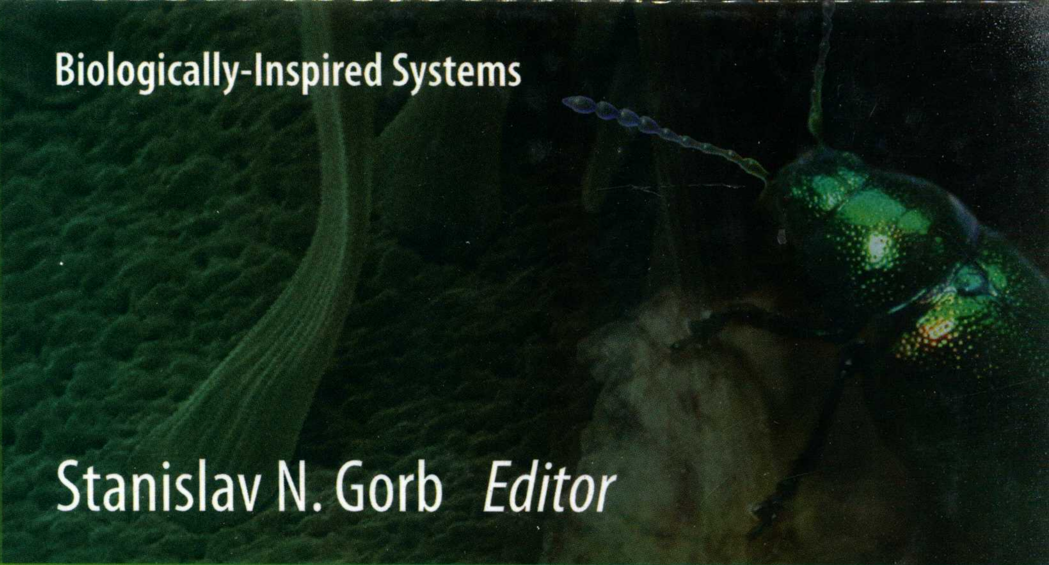
Biologically-Inspired Systems



Stanislav N. Gorb *Editor*

Adhesion and Friction in Biological Systems

Springer

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Cover illustration: The green dock beetle *Gastrophysa viridula* on its host plant. In the background: scanning electron microscopy image of adhesive hairs of the beetle leg in contact with the rough surface.

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Adhesion and Friction in Biological Systems

BIOLOGICALLY-INSPIRED SYSTEMS

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Series Preface

Structure and function of biological systems as inspiration for technical developments
Throughout evolution, organisms have evolved an immense variety of materials, structures, and systems. This book series deals with topics related to structure-function relationships in diverse biological systems and shows how knowledge from biology can be used for technical developments (bio-inspiration, biomimetics).

Preface

Biotribology: Diversity matters

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The majority of edited books on biotribology deal with a diversity of approaches for microscopical, mechanical, and tribological characterization of surfaces, but most of them are restricted to very few model systems dealing with different materials of the human body or implants. However, we have to keep in mind that *Homo sapiens* is only one of 13–14 million of estimated species of organisms, and interesting adhesion- and friction-related contact problems can be found everywhere in Nature. Gecko foot, snake skin, insect joints are just a few striking examples from a tremendous diversity of biological surfaces and systems that remain rather poorly studied, when compared to medically relevant biotribosystems.

In 2001, with the publication of "*Biological Micro- and Nanotribology: Nature's Solutions*" by Scherge and Gorb we tried to set a new trend in studying tribological phenomena in diverse biological systems. The book described a few biomechanical systems with frictional surfaces or fluid secretions involved in attaching parts of the body to each other, or the entire organism to a substrate. Such systems have fascinated microscopists for centuries, but only recently the use of novel experimental and microscopical methods enabled the functioning of at least some of these systems to be really understood.

Since then, a large amount of scientific publications devoted to biotribology and bioadhesion of various biological systems have appeared in the literature. In the meantime, research on frictional and adhesive properties of very diverse biological surfaces and interfaces became quite a broad field in research on the frontier between tribology and biology (see Figure, Gebeshuber et al., 2009). During the past decade in my group, which was initially called *Biological Microtribology Group* at the Max Planck Institute of Developmental Biology, Tübingen, Germany (1999–2002), later *Evolutionary Biomaterials Group* at the Max Planck Institute for Metals Research, Stuttgart, Germany (2002–2008), and finally *Functional Morphology and Biomechanics* at the University of Kiel, Germany (2008–), we concentrated on studies of biological adhesive systems and their biomimetic prototypes, as well as on biological materials with specialized frictional properties. We followed an approach in which various microscopy methods, such as light microscopy, white-light interferometry, SEM, Cryo-SEM, TEM, and AFM were combined with force measurement techniques at the macro-, micro- and nanoscale. These studies resulted in numerous publications which, depending on the major experimental approach, were published in biological, materials science, engineering, or physical journals accessed mostly by members of particular scientific communities. Our idea was to collect these publications into one volume to provide potential readers with an overview of systems, phenomena, and approaches on a variety of non-human friction and adhesive systems in biology.

This book is a collection of experimental studies from my group originally published in various Springer journals. These studies that were carried out in collaboration with coworkers within the group and with numerous colleagues from other institutions worldwide, demonstrate structure-function relationships in biological systems having particular surface specialization to increase/decrease friction and adhesion. Studies on snake skin, adhesive pads of insects and spiders, wing-interlocking devices, and sticky mouthparts of insects, as well as anti-adhesive and adhesive surfaces of plants are included in the present volume containing four main subsections: (1) adhesion, (2) friction, (3) attachment devices, (4) attachment-related behavior.

In the first section, four different adhesion-related systems are discussed. Two of them deal with the enhancement of adhesion forces: moist mouthparts of beetles that are specialized for the collection of pollen grain (Karolyi et al.) and orthopteran attachment pads demonstrating different adhesive properties, depending on their mechanical properties (Perez Goodwyn et al). The third system, represented in this section by epicuticular waxes of plants (Gorb et al.), is specialized in reducing adhesion of insect attachment devices. Epicuticular wax crystals might be fractured by insect adhesive organs and contaminate the latter, depending on crystal geometry (Borodich et al.). The exact function of the fourth system, which is the covering wing surface in the female Colorado potato beetle, remains unresolved. However, this is an important study characterizing the female surface to which males adhere while mating. It is known that the covering wing surfaces of the female may play a role in sexual selection due to their different adhesive properties (Voigt et al.).

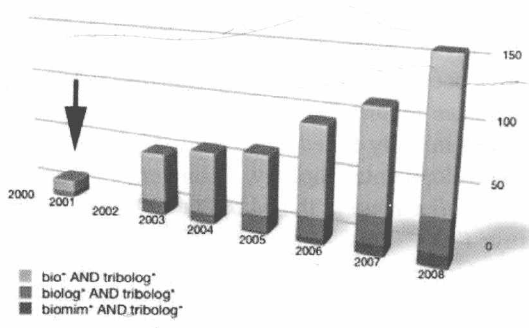

Figure. Number of publications on biotribology in the period from 2000 to 2008 (from Gebeshuber et al., 2009). Arrow indicates the publication year of the book *Biological Micro- and Nanotribology: Nature's Solutions* (Scherge and Gorb, 2001).

The second section, starting with some general statements on the microtribology of biological materials (Scherge and Gorb), is devoted to friction in various organisms such as spiders, insects, and snakes. The first two chapters deal with friction-related phenomena in arthropods, such as friction anisotropy in spider attachment pads (Niederegger and Gorb), and friction reduction mechanism in sliding joints between the fore-wing and hind-wing in bugs (Perez Goodwyn and Gorb). In the two last contributions of the section, the surface structure, frictional properties (Berthé et al.), hardness, and effective elasticity modulus (Klein et al.) of the snake skin are described.

The third section contains four papers on insect attachment devices starting with a broad overview on locomotory attachment pads of hexapods (Gorb and Beutel). The next chapter shows the relationship between the ultrastructural architecture and mechanical properties of attachment pads in the grasshopper (Gorb et al.). In chapter three, Niederegger et al. have applied a combination of methods to visualize contact behavior of adhesive setae in the fly attachment pad. Finally, an unusual micro-Velcro mechanism, called mid-coxal prong, is described for Brachycera (Diptera) in the fourth chapter of the section (Frantsevich and Gorb).

How do insects stick and how do plants prevent this? Four chapters in the final section deal with insect locomotory behavior that is related to the attachment ability of insects to different surfaces and structures of their natural terrain, which is mainly represented by plant surfaces. The general overview of surface related insect-plant relationships is provided in the first chapter (Gorb and Gorb). Insect walking techniques on thin stems are described and catalogued by Gladun and Gorb. Mirid bug locomotion on sticky plant terrain is studied in detail in the two final chapters (Voigt et al.; Voigt and Gorb).

Additionally, numerous experimental methods for characterizing tribological properties of biological surfaces at macro-, micro-, and nanoscale levels are demonstrated in the book. We are confident that this book will be an example of interdisciplinary science in action, combining approaches from biology, physics, engineering, tribology, and materials science. This work may potentially be of interest to both engineers and physicists working with biological systems, as well as to biologists studying friction and adhesion. Since biological surfaces have a great potential for technological ideas that could be used in developing new materials and systems, inspirations from biology reported here may also be used for biomimetics purposes.

I am most grateful to L.I. Frantsevich (Schmalhausen Institute of Zoology, Kiev, Ukraine), who, as my first supervisor, recognized the strong potential of studies on biological adhesion and friction and co-authored one of the chapters in this book. U. Schwarz (Max Planck Institute of Developmental Biology, Tübingen, Germany) strongly supported this branch of research during my stay at his institute. I have greatly benefited from collaboration with M. Scherge (MikroTribologie Centrum µTC, Fraunhofer-Institut für Werkstoffmechanik IWM, Freiburg, Germany), who, with his strong experience in microtribology, provided much advice on the measurements of small forces, development of equipment for micro- and nanotribology research (see his co-authored chapters in the book), and inspired me to co-author the book on *Biological Micro- and Nanotribology* (Scherge and Gorb, 2001). Collaboration with R.G. Beutel (University of Jena, Germany) resulted in an integration of diverse structural information on attachment pads of insects into a solid evolutionary context. I also wish to thank H. Schwarz (co-author of one of the chapters), J. Berger, I. Zimmermann, V. Kastner, B. Sailer, G. Scheer (Max Planck Institute of Developmental Biology, Tübingen), who helped with various microscopy techniques that contributed enormously to understanding the results obtained in mechanical tests.

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Contents

Adhesion

Friction

1. Adhesion

Introduction

Many flower visiting insects consume pollen and therefore possess characteristic mouthpart adaptations for taking up pollen grains from flowers. Insect mouthparts are highly integrated structural units, forming interfaces with the environment (Betz et al. 2003). They often possess dense bristles for harvesting small pollen grains, as well as structural modifications for the manipulation of ingested pollen. Pollen feeding has been recorded for a number of species from various families of beetles. Their prognathous mouthparts are adequate for feeding on nectar, pollen and flower petals (Krenn et al. 2005).

Pollen grains are encased by a hard and highly resistant outer hull, the exine (Johnson and Nicolson 2000) and single grains range from 10 to 200 μm in diameter (Stanley and Linskens 1974). The challenges confronting pollen feeders are the removal of the often dry pollen and gaining access to the encapsulated nutrition. Pollen is potentially a valuable food source for flower visiting insects since it contains a wide variety of lipids, carbohydrates and different protein concentrations (Johnson and Nicolson 2000).

Characteristic features of pollen feeding Coleoptera include an elongated maxillae with variously shaped combs and bristles that function as the primary pollen harvesting devices (Fuchs 1974). The mandibles, too, are particularly adapted for pollen manipulation and consist largely of the prostheca, a soft and usually bristly lobe, and the postmola which together knead the pollen (Schremmer 1961; Fuchs 1974; Nel and Scholtz 1990). The pollen grains are conveyed into the actual mouth opening by coordinated movements of the mouthparts (Fuchs 1974).

Although few investigations have been devoted to the mouthparts of flower visiting beetles, the functional anatomy of the mouthparts of the flower visiting rose beetle *Cetonia aurata* (Scarabaeidae) was previously studied in detail (Bürgis 1986a, b, 1987; Schremmer 1961). These studies concluded that the incisive part of the mandibles has lost its biting function, the mola part is responsible for crushing pollen and that the maxillae extend outward from their resting position under the clypeus and labrum with a semi-circular movement. Furthermore, the distal parts of the maxillae, i.e. the galeae, open laterally and extend forward. They reach into flowers and with their dense and wavy bristles sweep pollen grains into the cibarium (preoral cavity) during the retreating motion of the maxillae. Simultaneously, the laciniae with their short bristles convey any previously acquired pollen between the mandibles (Bürgis 1986a, b, 1987) (Fig. 1).

Despite the above mentioned studies on this frequently encountered European rose beetle, the full feeding mechanisms are not entirely understood. One open question concerns the possible interaction between pollen grains and the variously shaped bristles of the cuticle. The aim of this study was to examine the mouthparts of *C. aurata* under near natural conditions using a Cryo-scanning electron microscope. By examining rapidly frozen specimens which have not come in contact with liquids during preparation, this method allows the detection of pollen grains on mouthpart structures in their natural position. Any liquids naturally occurring on mouthpart surfaces will remain intact. The present examination represents the first application of this method for the visualisation of insect mouthparts. It demonstrated the presence of a liquid on the buccal surfaces of the mouthparts which compels us to new functional interpretations of mouthpart morphology and ultimately a deeper understanding of the pollen feeding mechanisms in this species. In addition, examination of the gut content should also provide insights in the role of different cuticular surfaces during feeding.

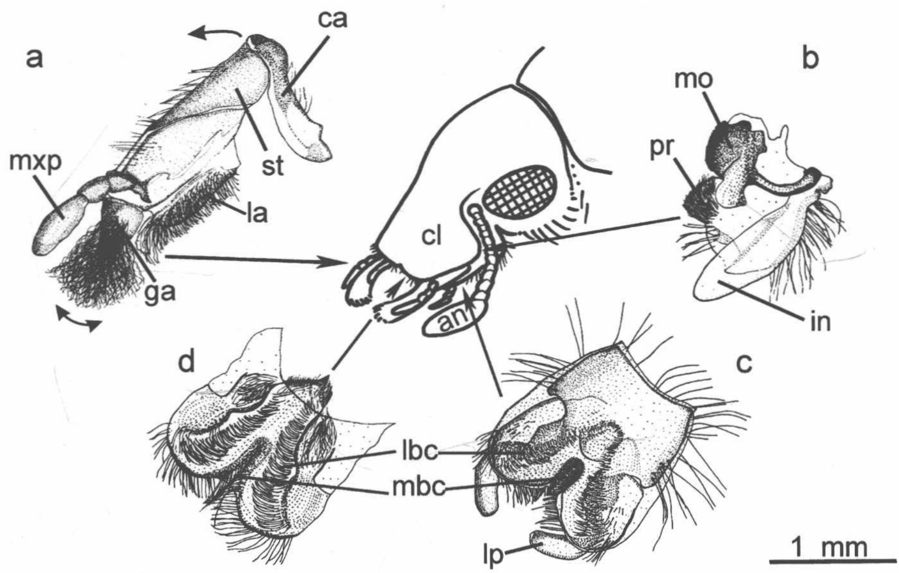

Fig. 1 Head and mouthparts of *Cetonia aurata* (Scarabaeidae). *Straight arrows* indicate position of mouthparts on the head; curved arrows indicate movements. **a** Maxilla swivels out laterally and extends forward; during its retraction the galea turns inward sweeping pollen to the lacinia. **b** Mandible with non-biting incisivus, prostheca and mola with pollen crushing surface. **c** Buccal side of labium with bristles crests. **d** Buccal side of labrum with bristle crests. The smooth dorsal side lies directly under the heavily sclerotised clypeus. an, antenna; ca, cardo; cl, clypeus; in, incisivus; ga, galea; la, lacinia; lbc, lateral bristle crest; lp, labial palpus; mbc, median bristle crest; mo, mola; mxp, maxillary palp; pr, prostheca; st, stipes. Bar scale applies to **a–d**

Material and methods

Cryo-scanning electron microscope (Cryo-SEM)

Specimens of the beetle *Cetonia aurata* (Coleoptera: Scarabaeidae, Cetoniinae), were collected from natural food plants in surroundings of Stuttgart-Vaihingen (southern Germany) and anaesthetized with CO_2 prior to preparation.

Heads were severed from the body of anaesthetized insects with the use of sharp razor blades, glued with Tissue-Tek O.C.T. fluid to brass stubs, and frozen in melting liquid nitrogen to prevent a gaseous phase building on the surface during the shock-freezing process. After a short sublimation procedure (about 3 min at the temperature difference −120 and −90°C), samples were sputter-coated in the frozen condition (temperature −140°C, thickness 6 nm) and observed in the SEM Hitachi S 4800 at accelerating voltage of 1–3 kV and temperature of −120°C. The method allowed us to visualise fluids located on the cuticle surface (Gorb 2006) and to study the surface of beetle mouthparts with high resolution and with a minimum of artefacts due to treatments by solvents and drying procedure.

Scanning electron microscope

The micrographs were taken using a conventional SEM (Philips XL 20) at the Institution of Cell Imaging and Ultrastructure Research (University of Vienna, Austria).

Beetles were collected from natural food plants in surroundings of Vienna. For the SEM, the specimens were rapidly frozen on flowers of *Crataegus monogyna* (Rosaceae) using a Cryo Freeze Aerosol (Agar Scientific Limited), stored in a can containing a nitrogen atmosphere, put in 100% ethanol, and finally stored at −54°C. To dissect mouthparts under a stereo microscope, the severed heads were then embedded in a molten wax-rosin mixture using a soldering tip (Star Tec ST 081).

All specimens were dehydrated in 100% ethanol and Hexamethyldisilazan (15 min) was used as an intermedium. After air drying over night, the objects were mounted on object holders using double-sided carbon-containing adhesive tape and sputter-coated with gold (300 s in an Agar sputtercoater B7340).

Gut examination

Beetles were collected from natural food plants in surroundings of Vienna and fixed in ethanol (70%). They were dissected under a stereo microscope, and their guts were removed. Parts of fore-, and hindgut were separately mounted in Glycerine on glass slides. Digital images were taken using a light optical microscope equipped with a digital photo camera Nikon CoolPix 950.

Results

Mouthparts and feeding mechanism

In *C. aurata*, the various structures of the mouthparts function together as a pollen harvesting and processing device. The cibarial roof is formed by the clypeus and the heart shaped labrum. This rather soft structure bears two lateral bristle combs, joining proximally to a v-shaped ridge and enfolding a median crest of bristles that perches on a median keel (Fig. 1d). The lateral bristles overlie the median part of the mandibles, while the bristly epipharynx lies between the mola surfaces. In Cryo-SEM micrographs all buccal bristles of the labrum are covered with a liquid. Pollen grains were found embedded in the liquid layer situated between moist bristles of the median crest. While the thinner, inner lateral bristles of the epipharynx point in the caudal direction, the outer lateral bristle rows align towards the median keel and the median bristles reach into the preoral cavity (Fig. 2a, b).

The distal parts of the maxillae, i.e., the galeae, represent the primary organs for pollen uptake (Figs. 1a, 2d–g). The Cryo-SEM study revealed that the long bristles on the tip of the galeae are completely saturated with fluid, forming a structure that can be functionally compared with a wet brush tip taking up pollen (Fig. 2e–g). Pollen grains were also found on the lacinia bristle crests. These bristles are likewise covered with a liquid to which the grains adhere, but they do not form a brush-like structure as described for the galeal bristles (not illustrated).

Conventional SEM micrographs revealed wavy bristles at the tip of the galea and a fine serration at the tip of otherwise smooth lacinia bristles (not illustrated). These structures presumably assist the pollen transport through the cibarium. It is not expected that they support the adherence of the liquid layer to the bristle's surface otherwise the entire shaft would be serrated.

The mandibles are composed of the thin blade-like incisivus which lies alongside the basal part of the maxilla, the soft and bristly prostheca and the well developed mola region (Fig. 1b). Cryo-SEM micrographs revealed that the bristles on the inner edge of the

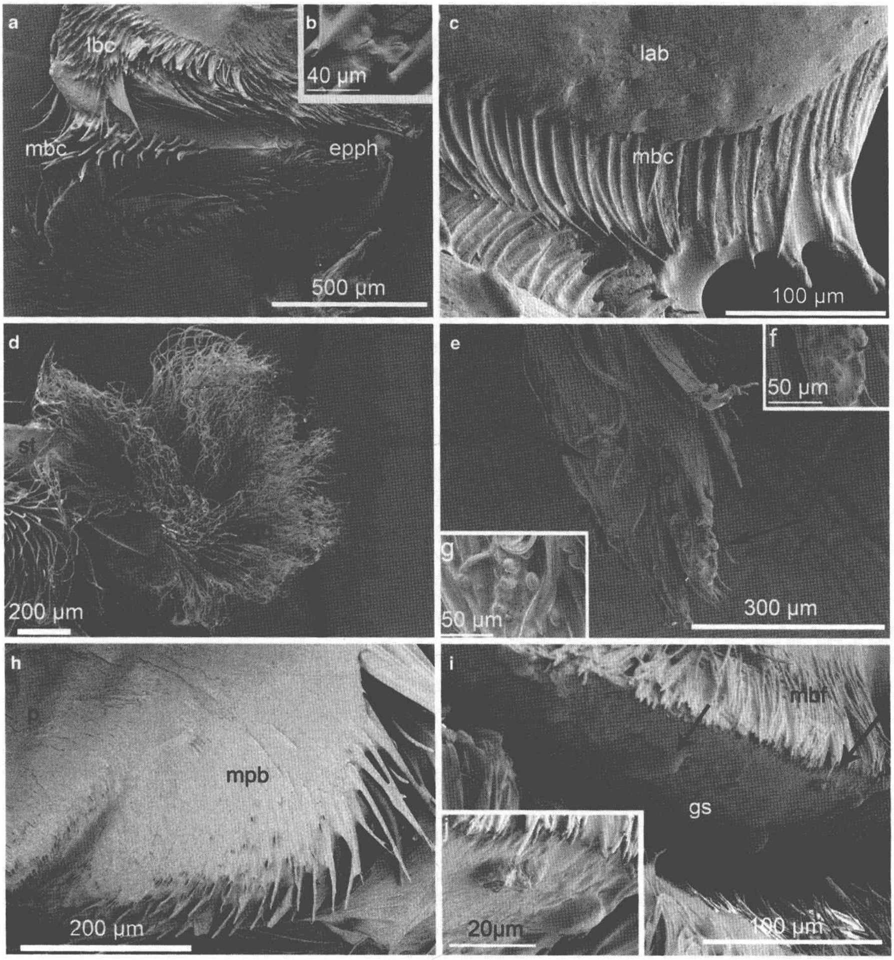

Fig. 2 Mouthparts of *Cetonia aurata*; Cryo-SEM micrographs (**a–c**, **e–j**) and SEM micrographs (**d**). **a** Ventral view of labrum with lateral bristle crests, median bristle crest and epipharynx. All bristles are covered with a fluid layer. **b** Detail of median bristle crest with pollen grains embedded in liquid layer (*arrows*). **c** Labium: dorsal view of moist median bristle crest covering the median depression. **d** Maxilla: wavy bristles of the galea forming a fan-like structure. **e** Galea tip, bristles forming a wet brush. Multiple pollen grains adhere to the liquid layer (*arrows*). **f, g** Pollen grains adhering to the moist galea tip. **h** Mandible: prostheca bristles with liquid layer, forming a pollen trapping and transport device. **i** Mandible: mola with moist grinding surface and wet bristle fringe. Single pollen grains are visible (*arrows*). **j** Detail view of the mola surface with a crushed and an intact pollen grain. epph, epipharynx; g, galea; gb, galea brush; gs, grinding surface; lab, labium; lbc, lateral bristle crest; mbc, median bristle crest; mbs, moist bristle fringe; mpb, moist prostheca bristles; p, prostheca; st, stipes

prostheca are entirely covered with liquid and thus adhere together, forming a large and dorsally vaulted region (Fig. 2h). Due to the convex shape it was concluded that this part of the mandible works like a pollen collecting receptacle when moving inside the cibarium. These structures clearly convey pollen to the mola region. This mandibular region with a sculptured chewing surface is covered with a liquid layer. Both crushed and intact pollen grains were detected to adhere to the fluid (Fig. 2i, j). The mola are surrounded by a fringe

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