Behavior and Its Neural Control in Gastropod Molluscs

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OXFORD
UNIVERSITY PRESS

2002

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Oxford New York
Auckland Bangkok Buenos Aires Cape Town Chennai
Dar es Salaam Delhi Florence Hong Kong Istanbul Karachi Kolkata
Kuala Lumpur Madrid Melbourne Mexico City Mumbai Nairobi
São Paulo Shanghai Singapore Taipei Tokyo Toronto

and an associated company in Berlin

Published by Oxford University Press, Inc
198 Madison Avenue, New York, New York 10016

www.oup.com

Library of Congress Cataloging-in-Publication Data
Chase, Ronald
Behavior and its neural control in gastropod molluscs/Ronald Chase.
p. cm.
Includes bibliographical references.
ISBN 0-19-511314-4
1. Gastropoda—Nervous system. 2. Gastropoda—Behavior. I. Title.
QL430.4.C455—2002
573.8′143—dc21 2001036788

9 8 7 6 5 4 3 2 1

Printed in the United States of America
on acid free paper

I dedicate this book to the memory of Jim Chase,
from whom I learned a love of scholarship.

Preface

This book is about the adaptive behaviors and the efficient brains of snails and slugs. Unfortunately, too many people think that the behavior of slugs is only sluggish, and that snails have no brains at all. In fact, gastropod molluscs are as interesting as any other animal, and we have a detailed understanding of how their brains work. During the past quarter century, there has been a tremendous increase in our knowledge about gastropods, their behavior and their neurobiology. This book is intended to review and celebrate that accomplishment.

It is the possibility of gaining detailed knowledge of both behavior and the nervous system that makes gastropod molluscs such attractive animals for biological investigation. Each subject, behavior and the nervous system, can be advantageously studied in gastropods. The behavior of gastropods, for example, is intermediate in complexity compared to other animals. It is neither as complex as the behavior of mammals and insects, nor as simple as the behavior of worms and echinoderms. Also, it has the added feature of being slow, which can sometimes be convenient for the scientist. All things considered, the behavior of gastropods is sufficiently complex to be interesting but not so complex as to be horribly difficult to describe. Similarly, the gastropod central nervous system contains more neurons than does the nervous system of worms and rotifers, for example, but it has fewer neurons than the brains of crustaceans and mammals. The precise number of neurons present in any central nervous system is difficult to determine, but generally speaking, estimates for the three major gastropod groups are in the range of 50,000–200,000 neurons for prosobranch species, 4,000–20,000 neurons for opisthobranch species, and 5,000–100,000 for pulmonate species. A complicating factor for investigators is the presence of a peripheral nervous system, which is difficult to access, but this problem is offset by the opportunity to work with central neurons of exceptionally large size.

The study of molluscan behavior from a neurobiological perspective has many early precedents but only a true beginning around 1965. In the writings of Artistotle and Pliny, we see evidence that the ancients paid attention to the most obvious behaviors, such as hibernation in land snails and inking in *Aplysia.* Illustrations of snail shells commonly appeared in books about natural history published in the 17th century. The first illustrated guide to shells, the *Historia Conchyliorum*, was published by Martin Lister in England in 1685. Interest in the animals that lived inside the shells grew significantly after publication of Linnaeus's great work of classification in 1758. However, it was not until the end of the 19th century that anything was known about the nervous system and, surprisingly, at that time the study of molluscan behavior had hardly progressed since the days of Artistotle.

The state of knowledge of molluscan behavior late in the 19th century can be illustrated by quoting a passage from Charles Darwin's *The Descent of Man and Selection in Relation to Sex.* Although Darwin himself was a reliable observer, and not usually taken in by imaginary animal stories, he accepted as true an account of snail behavior communicated to him by an acquaintance, one Mr. Lonsdale. The latter individual had placed two snails, *Helix pomatia,* into a garden that was evidently ill-provided with food. One of the snails was strong and healthy whereas the other snail was weak. From evidence of a slime trail, Mr. Lonsdale induced that the strong snail at one point had climbed over a wall and into an adjacent garden. Now quoting Darwin:

> "Mr. Lonsdale concluded that it [the healthy snail] had deserted its sickly mate; but after an absence of twenty-four hours it returned, and apparently communicated the result of its successful exploration, for both then started along the same track and disappeared over the wall" (Darwin, 1871, p. 325)

What is remarkable here is that Darwin accepted not only the observations but also the interpretation, namely that the healthy snail returned to help its mate because of a sense of "permanent attachment." Today, we know that there is no pair bonding in snails, and no ability to communicate in the indicated manner, although snails do commonly follow slime trails. Eleven years after Darwin published this story, George Romanes recounted it in his own book, *Animal Intelligence.* Although Romanes wished for an experimental replication, he too was ready to accept the facts and interpretations as given by Lonsdale and Darwin.

Another interesting account, in this case obtained third-hand, was reported by the eminent American malacologist William Healy Dall in an article entitled "Intelligence in a snail" (1881). The informant's sister had a pet snail that was remarkable not only for the fact that it could hear voices, but also because it could distinguish its owner's voice from the voices of other persons. It seems that the snail would crawl to the owner when spoken to, but it withdrew into its shell when spoken to by anyone else. After relating what the woman had told him, Dall writes that the facts may be "surprising" but he had no doubts of their "substantial accuracy."

While some zoologists were concerned with the mental capacities of snails, others were taking advantage of improved microscopic techniques to learn about the nervous system. One of the first and most significant discoveries was the presence of giant nerve cells. This was reported at least as early as 1863, when Buchholz reported them in freshwater snails (see Bullock, 1965). The anatomical papers that began to appear at this time and that continued to be produced in great numbers for another half century are full of detailed descriptions and beautiful illustrations. Although few tools were yet at hand with which to study function—mostly scalpels for producing lesions—the knowledge of neuroanatomy gained from these works laid the foundation for modern neurophysiological studies. However, much of it remained unknown or inaccessible to English-speaking scientists until the publication in 1965 of the monumental two-volume treatise on invertebrate nervous systems written by Theodore Bullock and G. Adrian Horridge. Bullock's chapter on gastropod molluscs alone contains citations to more than 750 papers, many of which are written in languages other than English. Still, the frontier of physiological neurobiology was yet to be truly breached.

The years just preceding and just following 1960 saw the establishment of several research laboratories that were to play key roles in the subsequent blossoming of molluscan neurobiology. In each case, individual scientists were at the center of the new laboratories. Professor K.S. Koshtoyants at Moscow State University trained three students who established laboratories in Eastern Europe: Dimitri Sakharov in Moscow, and Janos Salánki and Katalin Rózsa in Tihany, Hungary. Meanwhile, other laboratories devoted to the study of gastropod neurobiology were set up by Professor J. Lever in Amsterdam, by Gerald Kerkut in Southampton, England, and by Ladislav Tauc in Paris. Transplantation of these efforts to North America occurred following cross-fertilizations in Tauc's laboratory.

When Eric Kandel visited Tauc's laboratory in 1961, he was a psychiatric resident and research associate at the Massachusetts Mental Health Center in Boston. Having already experienced the frustration of neurophysiological research on the mammalian brain, he was open to new opportunities. He learned about the giant nerve cells of gastropod molluscs from a lecture given by Angelique Arvanitaki at the National Institutes of Health. As a biology student, working at a Mediterranean field station in the early 1940s, Arvanitaki had discovered the potential of *Aplysia*'s giant nerve cells for neurophysiological research. She shared her knowledge and enthusiasm for these cells with Tauc, who then passed it on to Kandel when he arrived for collaborative work. After Kandel's return to Boston, he established his own laboratory devoted to the study of *Aplysia*. Mixing talk therapy upstairs with slug dissections downstairs, Kandel quickly became acquainted with many identifiable neurons in the abdominal ganglion, and he began to study their roles in the control of behavior. Meanwhile, another American, Felix Strumwasser, had also heard Arvanitaki's lecture and he had also visited Tauc's Parisian laboratory. Thus, with Kandel established in Boston, and Strumwasser at the California Institute of Technology, an enthusiasm for sea slug neurobiology began to sweep across America in the early 1970s. Whereas Kandel's research was specifically directed towards discovering the mechanisms of learning and memory, with its ultimate application to psychiatry, his work led many scientists to realize that gastropod molluscs need to be understood in their own biological context and for their own inherent interest.

The accelerating attention paid to gastropods as subjects for neurobiology during the decades of the 1960s and 1970s was part of a broader effort to discover and exploit animal models that were simpler to understand and more convenient to work with than mammals. Although insects, lower vertebrates and other groups were also being investigated for these purposes, the large size of molluscan neurons was especially attractive to physiologists who required stable intracellular recordings for biophysical work. Others were attracted to giant nerve cells because they allowed for replicable experiments on the role of individual neurons in the control of behavior. Furthermore, and importantly, the rapid success of Kandel and his colleagues in accounting for certain simple behaviors in terms of identified cells, such as in defensive withdrawal reflexes and ink ejection, encouraged a similar approach in a variety of gastropod species besides *Aplysia* that also have large neurons. Kandel summarized the results obtained by his group and others in two remarkable books published in 1976 and 1979. Although the first book is broader in its approach than the second, both books essentially review progress in understanding the neural control of behavior in *Aplysia*. With the exception of a two-volume collection of reviews edited by Dennis Willows (see Mpitsos and Lukowiak, 1985; Dorsett, 1986), there has been no comprehensive review of gastropod neurobiology since Kandel's works.

In the present book, I include material pertaining to all groups of gastropod molluscs, and I cover both behavior and neurobiology. While the scope is therefore broad, I focus on recent discoveries that explain the neural control of behavior. My approach to the subject assumes that behavior is controlled by the cellular connections between specific neurons that operate within knowable circuits. In this respect, I accept the reductionistic premise that has fueled the recent growth of the field. Whether this approach will continue to be successful remains to be seen, but there is no denying the tremendous amount of knowledge that has so far been generated by adopting this approach, as evidenced in this book. In order to focus on the structure and function of the nervous system, I have not gone deeply into biophysics or molecular aspects, nor have I given justice to the extensive literature on chemical neuroanatomy that reports the use of immunohistochemical methods to localize neurotransmitters and neuromodulators within nervous systems. I have only included mention of these subjects in so far as they bear directly on the mechanisms of behavioral control.

I hope that curious persons of diverse backgrounds will read this book. Those readers who have a prior knowledge of either molluscs or neurobiology, but not necessarily of both, will benefit most readily. However, anyone with a basic biology education should be able to understand the book. Because I assume that the reader already knows about neural signals, synaptic transmission, and so forth, I make no attempt to teach neurophysiology. As an introduction to the subject, and for subsequent reference, I begin the book with illustrations of the gastropod species that are most commonly studied in neurobiological research, together with drawings of their central nervous systems. The first four chapters should be particularly helpful to persons with backgrounds in vertebrate neuroscience, since they cover the broader aspects of molluscan biology and draw attention to the special features of the gastropod nervous system. Each of the remaining six chapters treats a different type of behavior. In general, I begin these later chapters by reviewing how the demands for behavior differ in diverse taxa, and how the adaptations differ. I then review progress in understanding the mechanisms of neural control, emphasizing cases in which control can be attributed to identified neurons and identified neural circuits.

Because the scope of the subject matter is so broad, it has not been possible to include references to all pertinent publications. Although the list of publications is long, it is still greatly abbreviated relative to the actual literature that it represents. I regret having to omit mention of so many worthy authors. Nevertheless, I have tried to provide enough references to key papers to allow readers to quickly find additional publications on any subject that is mentioned in the text. With computer-based resources, it is easy to generate a long list of references on any chosen subject, but the search is greatly facilitated if one can start with a key reference. For this reason, and because credit should be given to those to whom it is due, I have usually cited the original and central publication in respect to all major findings. However, in some cases, for the sake of economy, I have had to cite just a single paper in reference to several related findings, or to refer to secondary sources. One way or another, I have tried to make the list of publications useful to those who wish to find out more.

I would like to thank several people and institutions that contributed to the completion of the book: Yutaka Nishioka gave me the initial impetus; McGill University provided the opportunity, and my colleagues in the Department of Biology provided the requisite intellectual climate; my students, postdocs, and assistants throughout the years helped to make my own research on "snail brains" successful and fun; thanks to them for their enthusiasm, and thanks to the Natural Sciences and Engineering Research Council of Canada (NSERC) for consistent funding. The McGill University libraries either had what I needed or they got it; special thanks to Eleanor MacLean of the Blacker-Wood Library for her expertise. Thanks also to Kirk Jensen, my editor at Oxford University Press, for assisting at all stages in the preparation of the book. The illustrations shown in Figures I–VII were drawn by the talented and gracious artist Josée Morin. Wayne Sossin lent me one of his animals for the cover photograph. Finally, I am very grateful to my expert reviewers who provided feedback on earlier version of the manuscript. Irving Kupfermann read the entire manuscript and gave me many useful suggestions. Sadly, Irving died shortly before this book was published. His originality, vast knowledge and knack for discovery were elements that contributed in important ways to the science reviewed here. Other persons who read and criticized one or more chapters are Paul Benjamin, Rhanor Gillette, John Koester, Jon Jacklet, Louise Page, David Rogers, Rich Satterlie, Andries ter Maat, and Terry Walters. Many thanks to all of you.

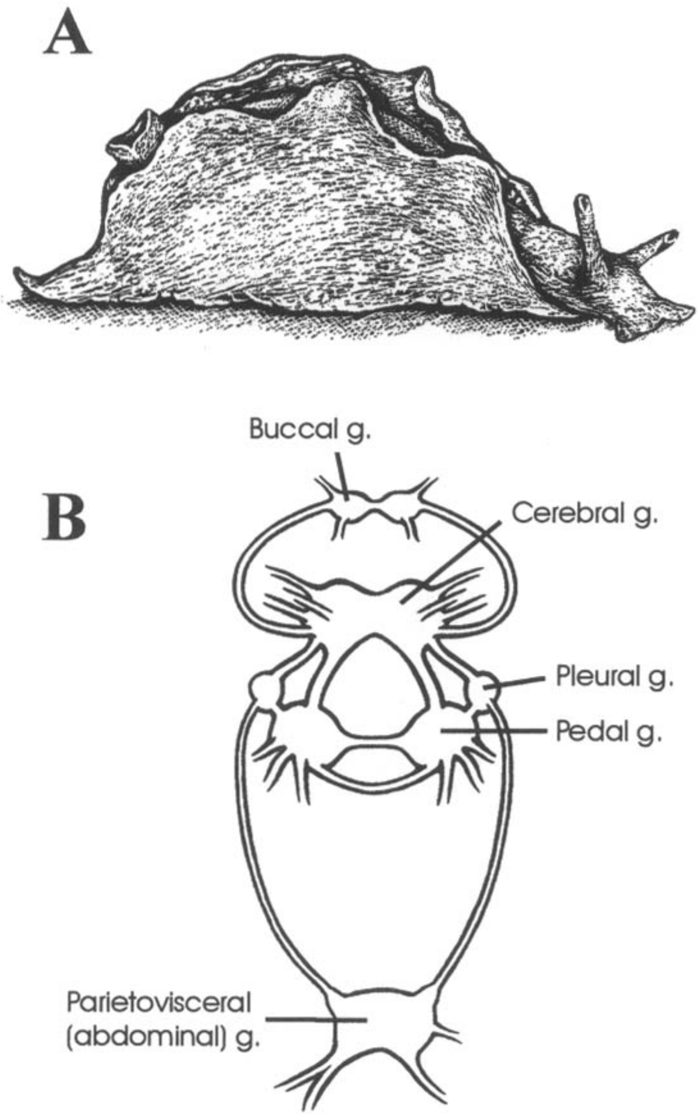

Figure 1. *Aplysia californica* (Opisthobranchia, Anaspidea). (A) There are about 37 species of *Aplysia* distributed worldwide, mostly in warm oceans. Since the time of Pliny, in the first century A.D., *Aplysia* has been known as the "sea hare" because of its resemblance to the terrestrial mammal. *A. californica* is found in the intertidal and subtidal zones from central California to northern Mexico. It feeds primarily on red algae. A typical adult is 20 cm in length and weighs about 500 g. (B) The central nervous system comprises four paired ganglia plus the unpaired parietovisceral ganglion, or abdominal ganglion, which is the most intensely studied of all gastropod ganglia. The parietovisceral ganglion was formed by the fusion of four ancestral ganglia.

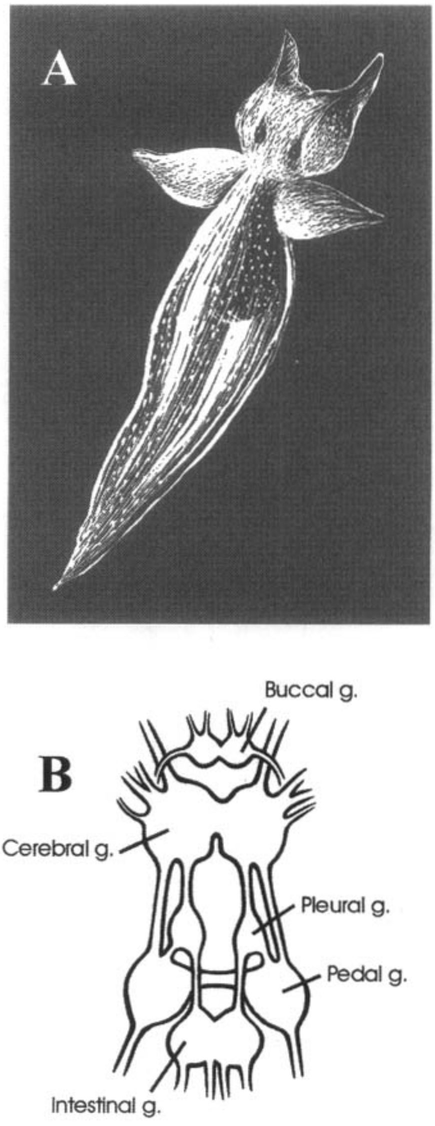

Figure II. *Clione limacina* (Opisthbranchia, Gymnosomata). (A) This shell-less animal, known as the "sea angel," lives its entire life drifting in the open ocean. Although it frequently swims in an upward vertical direction using highly muscularized "wings," it is unable to counteract the sea's currents. The adult body size ranges from about 20 mm in Puget Sound, state of Washington, to about 85 mm in the subarctic Atlantic Ocean. The species name is derived from the name of its exclusive prey, *Limacina*, which is a small planktonic snail (Thecosomata). Polar species feed on one species; temperate zone species feed on another species. (B) The central nervous system comprises five pairs of ganglia. The large posteriorly directed pedal nerve innervates the ipsilateral "wing."

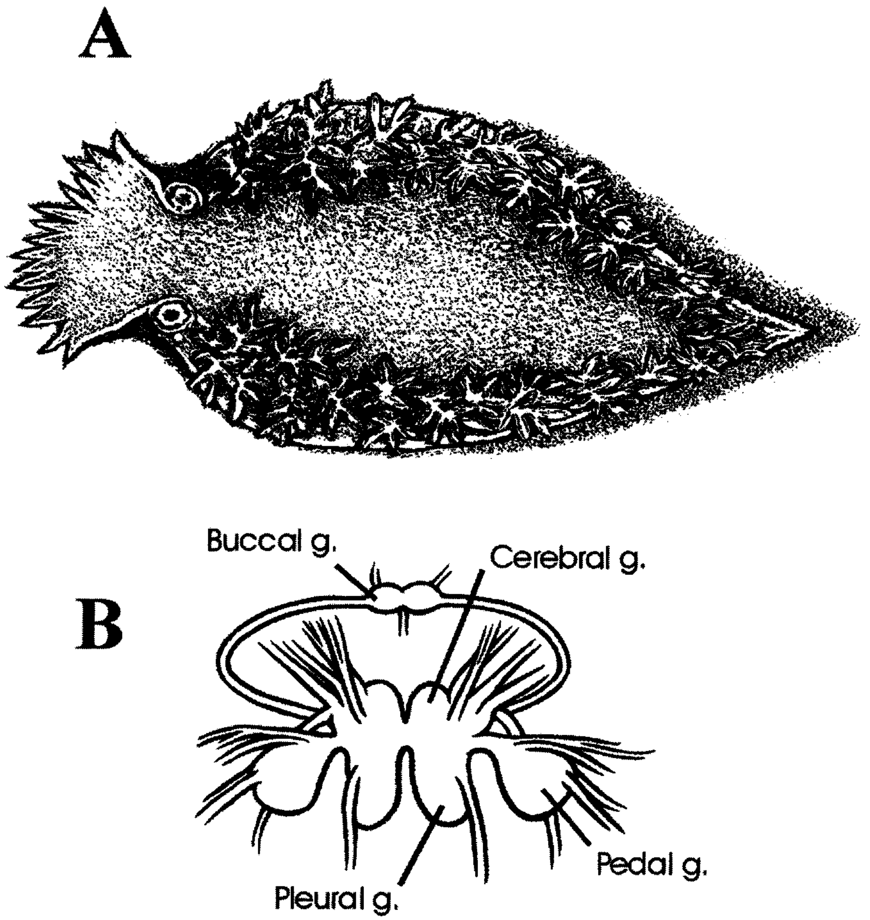

Figure III. *Tritonia diomedea* (Opisthobranchia, Nudibranchia). (A) This animal lives on soft sediments in the northern Pacific Ocean, typically in the near-shore subtidal beds of its prey organism, a coral sea pen. Commonly known as a "sea slug," *Tritonia* is usually found with a uniformly pink color. Prominent respiratory tufts (branchia) protrude from the margin of the dorsal surface. Specimens range in size up to 30 cm in length. (B) The central nervous system is extremely compact and symmetrical. Note the fused cerebral and pleural ganglia.

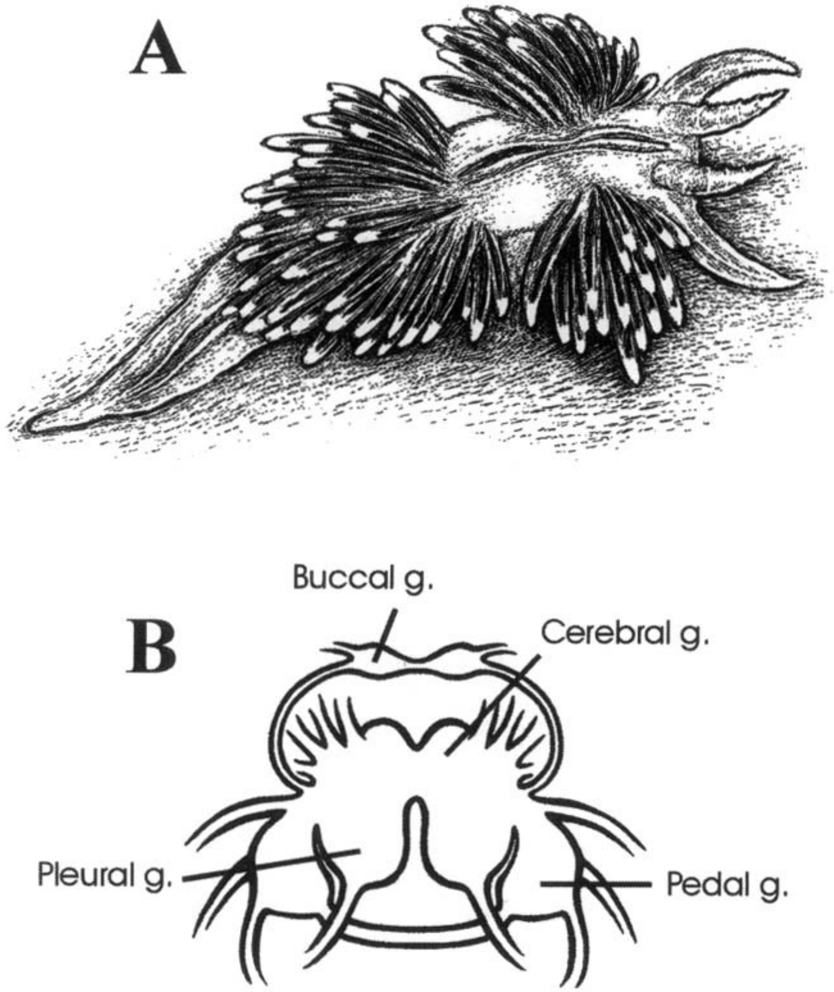

Figure IV. *Hermissenda crassicornis* (Opisthobranchia, Nudibranchia). (A) Like most nudibranchs, *Hermissenda* is strongly colored. Particularly striking is the bright orange stripe running down the anterior midline and the bright blue outlines around the oral tentacles. The respiratory tufts are modified as cerata containing diverticula of the digestive glands. *Hermissenda* is carnivorous; when it feeds on anemones or hydroids, the prey's nematocysts end up in the cerata, where they are recycled as defensive weapons. Thus, the coloration of *Hermissenda* is probably aposematic (i.e., it is a warning to would-be predators). The animal is found in rocky intertidal habitats from Alaska to Mexico, and also in Japan. The average adult size is 3–6 cm in length. (B) The central nervous system is highly condensed, as in *Tritonia*.

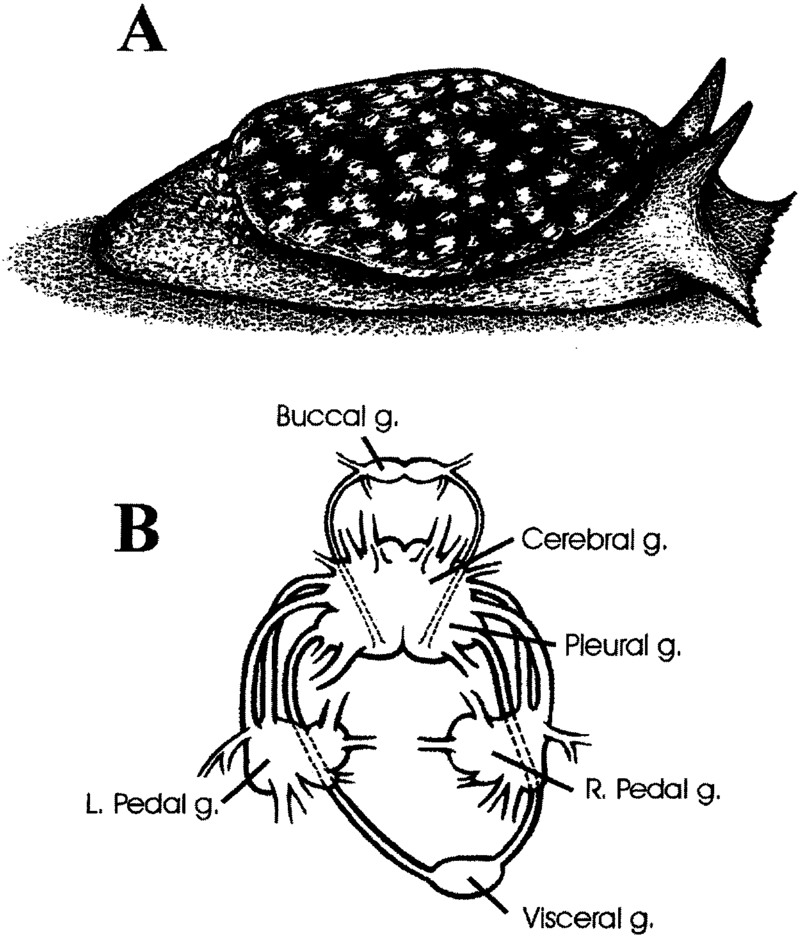

Figure V. *Pleurobranchaea californica* (Opisthobranchia, Notaspidea). (A) This animal lives along the Pacific coast of California at depths of 10–180 m. The prominent mantle covers an internal shell and a gill situated on the animal's right side. *Pleurobranchaea* is an indiscriminate carnivore that ingests its prey after capturing it with an explosive projection of the proboscis. When *Pleurobranchaea* itself is attacked, it releases a highly acidic mucus, pH 1–2. Sizes range from 15 to 50 cm. (B) Note that the fusion of the cerebral and pleural ganglia, and the close apposition of the left and right halves, creates a single cerebropleural ganglion.

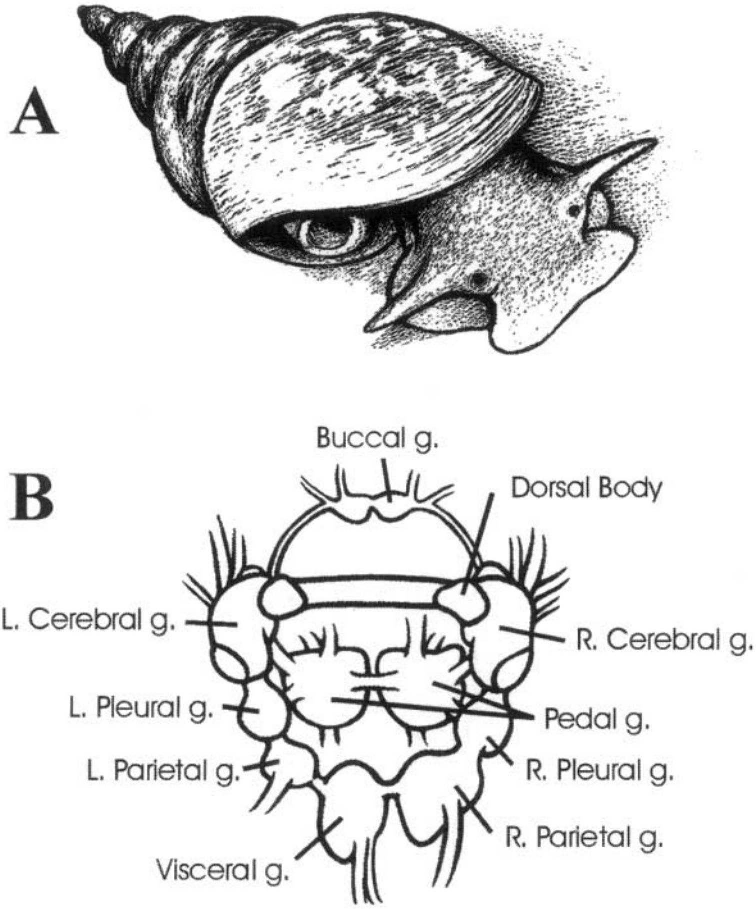

Figure VI. *Lymnaea stagnalis* (Pulmonata, Basommatophora). (A) This snail is a common inhabitant of fresh waters, especially where there is rich vegetation. It is found throughout Europe, North America, and northern Asia. The shell length ranges from 2 to 6 cm. There is just a single pair of tentacles, with an eye located at the base of each one. *Lymnaea* can respire through its skin while submerged, but it must occasionally surface to breathe air. For food, it rasps at plant leaves or grazes on thin algal films. (B) Although the dimensions of the central nervous system are small, the cells are highly pigmented and clearly visible. The dorsal bodies contain endocrine cells.

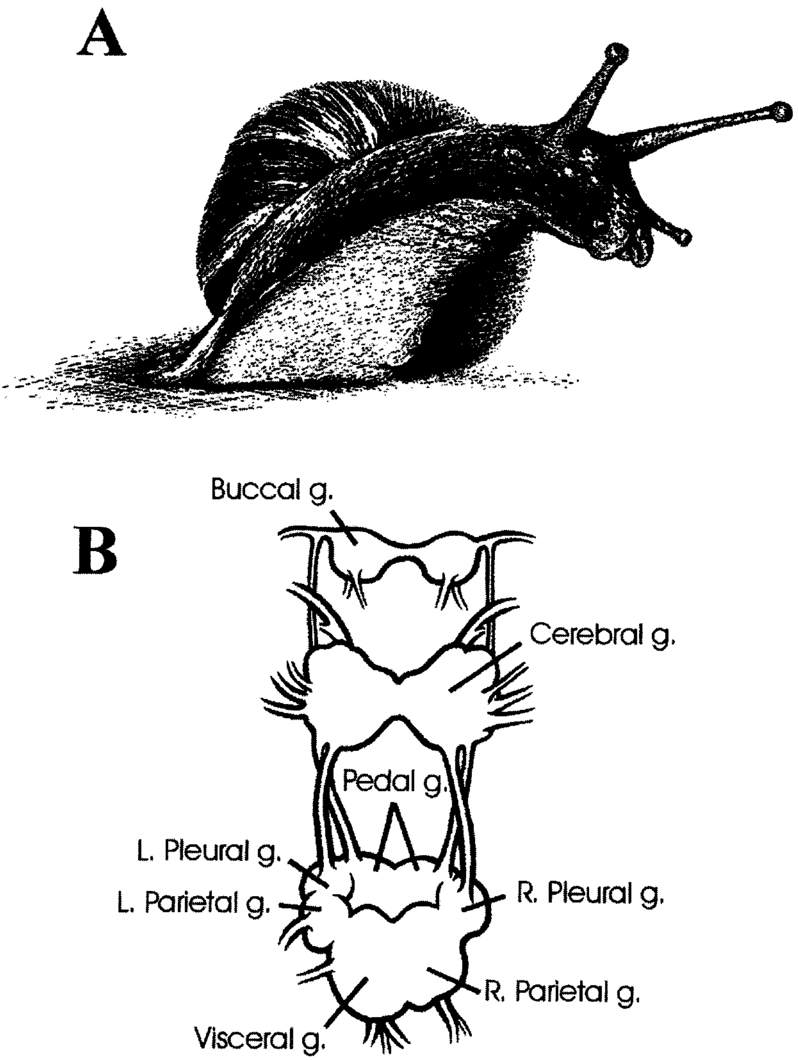

Figure VII. *Achatina fulica* (Pulmonata, Stylommatophora). (A) From its original home in East Africa, this terrestrial snail has spread to tropical and subtropical regions around the world. It is an agricultural pest of economic significance in many places, owing in part to its prodigious capacity to reproduce. Adults generally have shells about 6–10 cm long, but specimens as large as 18 cm have been found. All four tentacles carry an olfactory organ at their tips; the posterior pair also has an eye at the tip. (B) All ganglia, excepting the cerebrals and the buccals, lie beneath the esophagus where they form a ring around the cephalic aorta. Note that the right parietal ganglion is considerably larger than the left parietal ganglion.

Contents

Behavior & Its Neural Control in Gastropod Molluscs

1

The Gastropods

1.1. General Features

The phylum Mollusca is second only to the Anthropoda in number of species. The molluscan lineage has been extremely plastic, and a great variety of structural plans have appeared. Of the seven classes of molluscs (Fig. 1.1), the largest is Gastropoda, which accounts for about 80% of the extant fauna. Estimates of the total number of living gastropod species is at least 40,000 and perhaps more than 100,000, with about 13,000 named genera (Bieler, 1992); the range of uncertainty indicates our substantial ignorance of this major taxonomic group.

The word "gastropod" is something of a misnomer. The term was coined by George Baron Cuvier, in 1797, who was the first of the naturalist classifiers to notice that certain of the shell-bearing molluscs have much in common with other, shell-less molluscs. He gave the name "gastéropode," from the Greek words for stomach and foot, to all those molluscs that appeared similar. But what led Cuvier to construct this particular word? Probably, he picked up on the fact that these animals seem to crawl by means of their underparts, or bellies. It is true that the organ of locomotion in gastropods is located ventrally, where the human stomach is found, but it is not anatomically correct to say that gastropods crawl on their stomachs because the digestive tract of gastropods is mostly situated dorsally, within the shell, not near the foot. Nevertheless, apart from their roles in the naming of the class Gastropoda, the digestive system and the foot have played significant roles in the evolution of the so-named animals. For one, the evolutionary growth of the digestive gland forced a coiling of the dorsal part of the body, which is today evident in the spiral of snail shells. The shell itself is secondarily lost in slugs, but these animals still retain a visceral hump, which is an enlargement owing to the digestive gland. As for the foot, it has undergone frequent modifications to allow locomotion by different means, but since the foot is never very efficient as a locomotor force, the gastropods' early commitment to this form of locomotion accounts for their mostly sedentary habits.

The morphological traits (apomorphic characters) that define the Gastropoda in relation to sister taxa (Fig. 1.2) are the larval operculum, the shape of the larval shell and, most importantly, torsion, which is discussed below (Ponder and Lindberg, 1997). Also typical of gastropods is a clearly

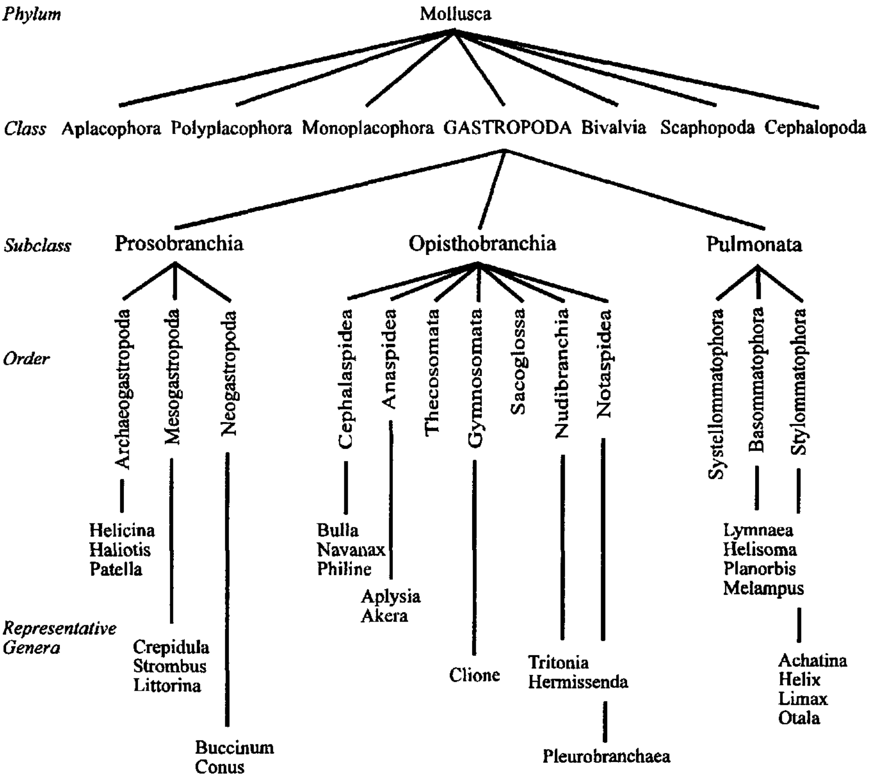

Figure 1.1. A traditional classification of the gastropod molluscs, based on morphological characters. Many points in the classification are controversial and subject to revision; one alternative is shown in Figure 1.2. The genera listed here are those that have been exploited for neurobiological research.

recognizable head bearing tentacles and eyes, a radula for rasping food, and nerve cells segregated in ganglia. However, none of these latter features is unique to gastropods, and they are presumed to be ancestral. The particular forms taken by gastropod species vary greatly, as do their sizes. Some species of terrestrial snails measure less than 1 mm in their longest dimension, whereas the sea slug *Aplysia vaccaria* can reach 990 mm in length and weigh 14 kg. Besides being morphologically diverse, gastropods also have diverse lifestyles. They are one of the few animal groups to successfully occupy marine, freshwater, and terrestrial habitats.

1.2. Torsion

Torsion is the single most distinguishing characteristic of the gastropods. Torsion refers only to the twisting of the body; it is entirely different

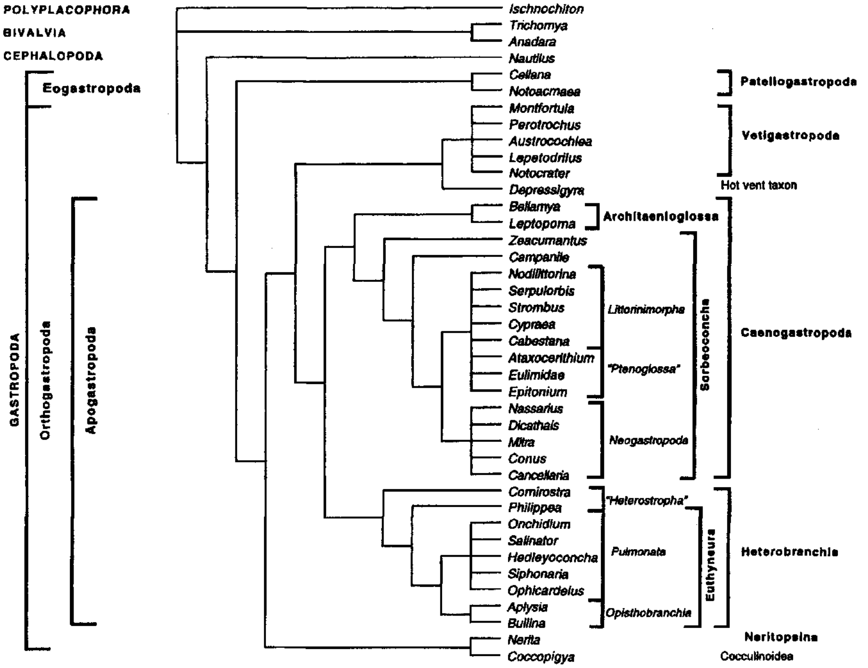

Figure 1.2. A proposed phylogeny of the gastropod molluscs, derived from a cladistic analysis of 117 morphological characters in 40 taxa (Ponder and Lindberg, 1997). Note that the primary division separates the Gastropoda into two major groups, Eogastropoda and Orthogastropoda, rather than the three subclasses shown in Figure 1.1. Sister groups to the Gastropoda are shown at the top. The listed taxa are those included in the work of Colgan et al. (2000), from whom the figure is taken. Using molecular sequence data, Colgan et al. (2000) found support for some aspects of the illustrated cladogram, but not for others.

from the spiraling of the shell. Fossil evidence suggests that early, non-twisted molluscs already had coiled shells. Conversely, some modern gastropods have uncoiled shells, or even no shell at all. The body plan of all modern gastropods undergoes torsion during some stage in the animal's development. Thus, torsion is a good example of ontogeny recapitulating phylogeny. But the significance of torsion goes beyond taxonomic classification, for it has profound effects on many body structures, including the nervous system.

To appreciate torsion, we need to imagine the body plan of the molluscs that existed before the advent of the gastropods. Such speculation rests on the study of extant non-gastropod molluscs, as well as the examination of fossil shells. The early molluscs were probably symmetrical, and they had a alimentary tract running straight down the center of the animal from the mouth at the anterior end to the anus at the posterior end (Fig. 1.3A). The anus was present within a space called the mantle cavity that lay beneath the overhanging shell. Also within the mantle cavity was a pair of ctenidia,

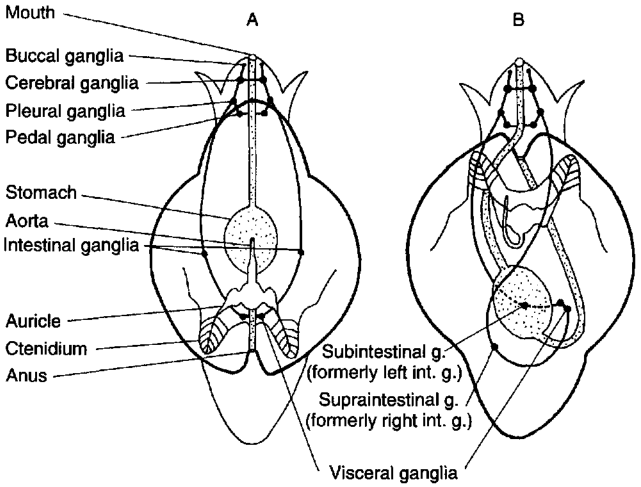

Figure 1.3. Schematic illustration of torsion and its effects on the arrangement of various organs. (A) A hypothetical molluscan ancestor of the gastropods, before torsion. (B) A hypothetical early gastropod, after torsion. From observations of developing gastropods, it is known that torsion occurs in a counterclockwise direction, thus accounting for the twisted appearance of the alimentary tract and the nervous system. The so-called visceral loop comprises the nerves and ganglia connecting the pair of pleural ganglia by way of the visceral ganglia. Some gastropods, particularly the opisthobranchs, have undergone a secondary detorsion during their evolution, so they appear more like (A) than (B).

or gills. At some point during the early Cambrian period, animals appeared in which the shell and the mantle cavity were rotated 180° counter-clockwise towards the anterior end (Fig. 1.3B). These animals were the first gastropods. After further changes, the mantle cavity came to lie at the anterior end, as it does today in most gastropods. The exceptions, notably the opisthobranchs, can be accounted for by the later occurrence of detorsion (Section 2.1).

One important consequence of torsion was the displacement of many interior organs. For example, before torsion the two heart auricles were located posterior to the ventricle, but after torsion they lay anterior to the ventricle (Fig. 1.3). The digestive tract became U-shaped, and some nervous ganglia, for example, the intestinal ganglia, moved to new positions. Primitively, there was a left and a right intestinal ganglion (Fig. 1.3A), but torsion caused the former left ganglion to move to the right of the former right ganglion (Fig. 1.3B). Since torsion occurs during development, either during the growth of nerves connecting the ganglia or after they have formed, the nervous system acquires a twisted appearance, a condition known as streptoneury. Note, in Figure 1.3B, that the left limb of the visceral nerve loop runs ventral to the alimentary tract, whereas the right limb is dorsal (see also Fig. 2.1). Contrasting to streptoneury is euthyneury, in which both limbs of the visceral loop run ventral to the alimentary tract and the loop as a whole is uncrossed; euthyneury is characteristic of some gastropod groups as the result of secondary evolutionary events (see Section 2.1).

Authorities disagree about why torsion occurred at all. A widely held view is that the torted condition benefits the larvae, which, in the ancestral forms, were pelagic. When the animals were untorted, the defensive withdrawal of exposed body parts was compromised because the withdrawal of such crucial structures as the head and the velum would have had to wait until the foot was withdrawn. After torsion, the head was able to retract first, and then withdrawal of the foot closed off the shell aperture to prevent access to the head. Also, the early withdrawal of the velum causes the larvae to stop swimming so that it falls in the water column and thus escapes the would-be predator. Although this makes a plausible story, others have pointed out that some pelagic molluscs (e.g., the lamillibranchs) are untorted and yet they survive predation.

Alternatively, it has been argued that torsion was an adaptation that allowed the gills better access to water flow, either to take advantage of flow patterns created by evolutionary changes in the shape of the coiled shell or simply to move the gills into the water current created by locomotion (Solem, 1974). Still another idea is that torsion brought the gills to the front end, where they can maximize benefits from small changes in the animal's orientation toward water currents (Morton, 1979).

If the reader has correctly understood the description of torsion and its consequences for the rearrangement of organs, it will be apparent that the early gastropods must have been defecating on their own heads. Indeed, the excrement would have been forced upon their heads and their gills by the water current. One way in which evolution looked after this problem was by selecting animals with holes in their shells so that the incoming water could be quickly swept out. Also, to reduce fouling of the gills by excrement, one of the two bilateral gills was lost, and the remaining gill was lateralized opposite the anus. In a subsequent development (the Pulmonata), the gill was entirely replaced by an internal respiratory structure, the lung.

1.3. Origins and Diversification of the Gastropoda

The Mollusca originated in the late Precambrian period or early Cambrian period about 600 million years ago (Solem, 1974; Runnegar and Pojeta, 1985). They came from a stock of flatworm creatures that also gave rise to the annelids. These ancestors were bilaterally symmetrical, unsegmented but serial in body plan, and acoelomate (i.e., lacking an internal body cavity lined with epithelium). They probably lived at the sandy bottom of the near shore, and they were probably carnivores. One major difference between these early molluscs and modern flatworms, or modern molluscs for that matter, is that the initial molluscs were tiny, measuring at most a few millimeters in length.

The gastropods originated in the early Cambrian period about 550 million years ago. Despite the existence of a fossil record, a great deal of uncertainty remains about the early events. Some authors maintain that the first gastropods were shaped like limpets (Haszprunar, 1988; i.e., with uncoiled shells), whereas others believe that they had coiled shells (Runnegar and Pojeta, 1985; Ponder and Lindberg, 1997). Workers in the latter camp disagree about whether the shells were coiled dextrally (to the right) or sinistrally (to the left). Because torsion is the defining character of the class Gastropoda, much effort has been given to finding the first of the torted molluscs. Possibly the first gastropods originated in the superfamily Bellerophontacea within the class Monoplacophora (Fig. 1.1). The bellerophonts became extinct about 250,000 years ago but they left a rich fossil record. Most of the shells have a perfect bilateral symmetry, as illustrated in Figure 1.4. The case for their inclusion in Gastropoda rests with an indentation that is present, in some forms, at the medial margin of the shell aperture (Fig. 1.4B). Some authorities (Knight et al., 1960; Haszprunar, 1988) believe that the slit is at the anterior end of the animal, and that it indicates movement of the anus and the mantle cavity from the rear of the animal to the front of the animal. As this implies a torsion of 180°, the bellerophonts would therefore qualify as gastropods. Other authorities find evidence in the fossils of bilaterally symmetrical muscle pairs, inconsistent with torsion, and they point out that a rotation of exactly 180° is unlikely; they believe the shell indentation is actually located at the posterior

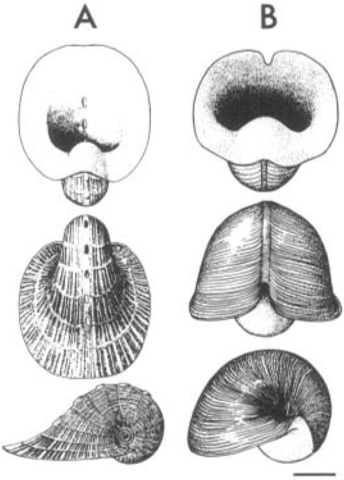

Figure 1.4. Two fossil examples of the extinct Bellerophontacea, believed by some authorities to be the first gastropod molluscs. (A) *Tremanotus*, (B) *Bellerophon*. Note the marginal slit at the midline of the shell aperture. If the slit represents the anus, and if it is present anteriorly in these specimens, then the shells indicate torsion, the hallmark of gastropod identity. Scale bar is 2.5 cm for (A), 0.5 cm for (B). From Knight et al. (1960) with permission.

end of the animal (Solem, 1974; Runnegar and Pojeta, 1985). According to the alternative view, the first gastropods came from a different group of monoplacophoran molluscs.

The classification of gastropods is unsettled. Early taxonomic classifications were based on the observed similarities and differences between taxa, whereas contemporary classifications are assumed to correspond to phylogenies, that is, they are based on hypotheses about the evolutionary descent of the taxa. Bieler (1992) attributes the difficulty of phylogenetic studies in this group of animals to "the long evolutionary history, the often rapid radiations, and the adaptation to many habitats by members of the same evolutionary line and to the same habitat by distantly related forms" (p. 315). The introduction of cladistic approaches, emphasizing the distinction between primitive versus derived characteristics, and computer-based parsimony methods have brought more power to the analyses, but the controversies have not abated. Similarly, molecular studies have not yet proven sufficiently compelling to quell uncertainties.

The traditional classification of the Gastropoda recognizes three subgroups of equal rank: Prosobranchia, Opisthobranchia, and Pulmonata (Fig. 1.1). This primary division of the class was originated by Milne-Edwards in 1848, based on the organization of the respiratory system. It later gained wide acceptance after publication of Thiele's treatise on Mollusca (1929–1931). However, as biologists examined more and more morphological characters, in greater and greater detail, the correctness of the traditional classification was increasingly questioned (see Bieler, 1992).

Following the publication of two influential papers by Haszprunar (1988) and Ponder and Lindberg (1997), a consensus was reached to reject any tripartite division of the Gastropoda. In these papers, convincing evidence was presented for the paraphyletic nature of the Prosobranchia, meaning that the taxa formerly included in this group do not have a common ancestor. This poses a problem for neurobiologists reading the earlier literature, which makes frequent references to prosobranchs. Therefore, in this book I will continue to refer to prosobranchs, as classified in Figure 1.1, but I will use quotation marks (e.g., "Prosobranchia") to signify that most taxonomists now reject the term. There is still utility in the term "prosobranch" as an indicator of gastropod molluscs that are neither opisthbranchs nor pulmonates.

It is generally agreed that the Opisthobranchia are paraphyletic or polyphyletic, but the Pulmonata, alone among the original three subclasses, are monophyletic (Haszprunar, 1988; Ponder and Lindberg, 1997; Colgan et al., 2000). These assertions must be taken as provisional, however, because neither the opisthobranchs nor the pulmonates have received much attention in recent analyses; the most intensely studied taxa belong to the "Prosobranchia." For example, Ponder and Lindberg (1997) used 40 taxa in total, but only four taxa from among Opisthobranchia and Pulmonata combined. In any case, I will use the terms Opisthobranchia and Pulmonata without quotation marks in this book.

A cladogram depicting the results of Ponder and Lindberg (1997) is shown in Figure 1.2. This is based on the most comprehensive cladistic analysis so far conducted, using 117 morphological characters, including those found in the shell, muscles, renopericardial system, reproductive system, digestive system, and nervous system. In comparison with the traditional scheme shown in Figure 1.1, the absence of "Prosobranchia" is evident. So, too, is the introduction of many new names to designate identified clades; many of these names are taken from earlier workers. It can be seen that the Gastropoda are monophyletic and comprise two clades, the small Eogastropoda and the much larger Orthogastropoda. The clades of principal interest to neurobiologists, Opisthobranchia and Pulmonata, are grouped as Euthyneura. Because the validity of the Euthyneura clade is widely supported (Haszprunar, 1988; Ponder and Lindberg, 1997; Colgan et al., 2000), it can be inferred that opisthobranchs and pulmonates descended from a common ancestor. It is noteworthy, however, that not all the conclusions of Ponder and Lindberg (1997) are well supported by a separate molecular study, which analyzed sequences from two segments of 28S rDNA and from the histone H3 gene (Colgan et al., 2000). This suggests that further revisions to the classification of the Gastropoda can be expected.

1.4. The "Prosobranchs"

The name prosobranch refers to the anterior position of the gills, which is the torted condition of early gastropods. Indeed, the primitive gastropod features are seen most clearly in the "prosobranchs." Contrastingly, many primitive features, including torsion, have been lost through secondary evolution in the opisthobranchs and the pulmonates. Most species of "prosobranchs" occupy marine habitats, but some species have adapted to freshwater and even terrestrial environments. Feeding strategies are very diverse. At the morphological level, major adaptive changes have occurred in the mantle cavity, the alimentary tract and the reproductive system. The treatise of Fretter and Graham (1994) provides a complete summary of this group, including many marvelous drawings.

Not surprisingly, there has been considerable controversy concerning the lower levels of classification within the "prosobranch" group. Traditionally, there are three orders: Archaeogastropoda, Mesogastropoda, and Neogastropoda (Fig. 1.1). As the names suggest, these groups were considered a progressive series. Haszprunar (1988) argued that there are just two "grades," the Archaeogastropoda and the Caenogastropoda (Caeno = combined), with the latter grade incorporating the Mesogastropoda and the Neogastropoda. However, the Archaeogastropoda are clearly paraphyletic and it is debatable whether the name should continue in use (Fretter and Graham, 1994; Ponder and Lindberg, 1997). Most of the taxa previously considered in this group are now assigned to the clade called Vetigastropoda (Fig. 1.2). Whereas the morphological analysis of Ponder and Lindberg (1997) strongly supports the Caenogastropoda, the molecular analysis of Colgan et al. (2000) only weakly supports it.

The Archaeogastropoda–Vetigastropoda possess many primitive characters including paired organs such as gills, auricles (diotocardian), kidneys, and osphradia. Figure 1.5 illustrates a representative of this group from the genus *Pleurotomaria*. According to Graham (1985), the earliest gastropods likely resembled this contemporary snail. By contrast, the Caenogastropoda have just one set of gills, one auricle (monotocardian), and one kidney, each of which is situated on the left side of the animal. The Mesogastropoda are probably paraphyletic (Graham, 1985; Ponder and Lindberg, 1997). The group includes many familiar seashore animals among which are the periwinkles (*Littorina*), the glossy cowries (Cypraeidea), and the large conches (*Strombus*). The Neogastropoda comprise exclusively marine snails, and they are probably monophyletic. The advancement of the carnivorous lifestyle in

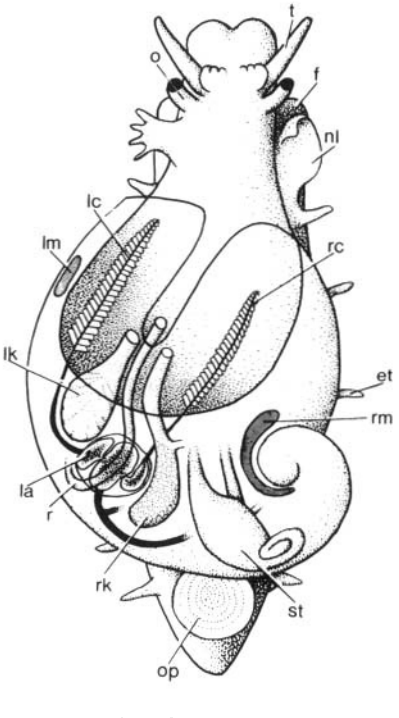

Figure 1.5. An archaeogastropod, *Pleurotomaria*. Many of the most primitive features of the class Gastropoda are evident in this modern snail. Note, for example, the paired gills, paired auricles, paired kidneys, and the slit at the anterior margin of the shell. The snail lives in the sea and feeds on detritus and thin algal growths. et, epipodial tentacle; f, foot; la, left auricle; lc, left ctenidium; lk, left kidney; lm, left shell muscle; nl, neck lobe; o, eye on eye stalk; op, operculum; r, rectum rc, right ctenidium; rk, right kidney; rm, right shell muscle; st, stomach; t, tentacle. From Graham (1985) with permission.

this group has produced some animals of special interest. Tropical snails of the family Volutacea burrow in the sand and smother prey with their large feet. One of these species, *Melo*, has a shell that is nearly a half-meter long. Many neogastropods (e.g., the muricids *Murex*, *Busycon*, *Buccinum*) are adept at boring holes in the shells of bivalves and gastropods. The toxoglossans (cone snails) have evolved a particularly dramatic style of feeding utilizing a poison that is injected into the prey via long, hollow, radular teeth. The poison is so potent, and its method of delivery so quick, that *Conus* snails are able to capture live fish.

The Heterostropha are something of a taxonomic dilemma. These animals seem to be mosaics of characters from several of the standard taxa. Their overall organization resembles the mesogastropods, but their radular teeth resemble the neogastropods. The shell, the osphradium, and the sperm are of types seen in the opisthobranchs. Currently, the Heterostropha are considered a separate group, but more aligned with the opisthobranchs and the pulmonates than with the "prosobranchs," primarily because they have an uncrossed (euthyneural) nervous system (Fig. 1.2).

1.5. The Opisthobranchs

The opisthobranchs (rear placed gills) appear in the fossil record at about the same time as the pulmonates in the late Carboniferous period, that is, some 300 million years after the first gastropods. The morphological feature that best distinguishes the opisthobranchs and the pulmonates from the "prosobranchs" is the uncrossed visceral nerve loop. The Opisthobranchia comprise the gastropod groups that remained in the marine habitat, while the Pulmonata comprise the groups that adapted to terrestrial and freshwater environments. It is possible that both the opisthobranchs and the pulmonates evolved from a common ancestor, as shown in Figure 1.2; alternatively, the pulmonates may have emerged from within the Opisthobranchia.

Apart from euthyneury, there are few features that absolutely distinguish the opisthobranchs from other gastropods. In their cladistic analysis involving 117 characters, Ponder and Lindberg (1997) identified only four character changes (apomorphies) associated with the emergence of the Opisthobranchia, among which is the presence of one marginal radular tooth rather than none, and the modification of the cephalic tentacles into rhinophores. However, only the families Aplysiidae and Acteonidae were included in the analysis. Other features generally characterizing opisthobranchs are the reduction or loss of the shell, and benthic habitats. Classification within the Opisthobranchia is difficult because of mosaicism and extensive parallel evolution, which precludes a phylogenetic reconstruction from strictly parsimonious approaches to morphological character analysis. Authorities differ greatly in their classifications at the ordinal level, with the total number of orders ranging from 8 to 14. The classification shown in Figure 1.1 assumes eight orders. Some authors recognize three higher level groups: the tectibranchs, which have a shell covering their gills; the nudibranchs, which have no shells (and often no gills); and the pteropods, which are planktonic. Although these groupings are not monophyletic, they are often used as indicators of type. When used in the older literature, the term tectibranch generally refers to all gastropods now classified as anaspids, cephalaspids, and notaspids, even though most anaspids have only vestigial shells. Each of the opisthobranch orders is briefly described below. Figures I–V (see pp. xii–xvi) illustrate the species most commonly studied by neurobiologists.

The most primitive morphological characters are found in the order Cephalaspidea. The name of this order refers to the presence of a protective head shield. The shell is small and internalized. Because the shell is also thin and bubble-like, these animals are known as "bulloids." Whereas all other opisthobranchs have become detorted, most of the cephalaspids are torted and they retain the primitive condition of a crossed visceral nerve loop. The foot is modifed as parapodial flaps, which serve as wedges for carrying the animal into or through the sand. Primitively, the cephalaspids have an open seminal groove, a non-retractile penis, and a common male/female genital pore.

The order Anaspidea (without shields) is characterized by a "migration" of the intestinal ganglia posteriorly and the fusion of these ganglia with the visceral ganglia (Section 2.2). The synonymous term, Aplysiomorpha, by which the order is also known, indicates the best known genus, *Aplysia* (Fig. I), and signals the importance of the group for neurobiologists. These animals are slug-like, with either a small shell or a vestigial shell. They have two tentacles and two modified tentacles, called rhinophores. In contrast to the Cephalaspidea, which are mainly burrowers, the Anaspidea are browsers that feed on surface algae. A gizzard grinds and strains the algae prior its ingestion. They are called "sea hares" on account of their feeding habits, as well as their appearance.

The Thecosomata and the Gymnosomata are together known as pteropods (winged feet). Both orders consist of planktonic swimmers, but they are otherwise quite different. The Thecosomata (encased bodies) have relatively primitive characters including a shell, feeding by ciliary capture of diatoms, and an operculum. The family Limacinidae consists of minute animals only a few millimeters in diameter with transparent spiraled shells, whereas the Cymbuliidae are more elongated and measure up to 5 cm. All these animals have very thin, but large, parapodia, which flap under muscular control and which give thecosomes their popular name, "sea butterflies." The Gymnosomata (naked bodies) are specialized as carnivores, and they feed mainly on thecosomes. They lack a shell, a mantle cavity, and gills; respiration is through the skin. Their bodies are streamlined for fast swimming, and they have a variety of specialized structures to aid in prey capture including, in different groups, hooks, suckers, sticky papillae, and sharp radular teeth. The widely distributed *Clione* (Fig. II) is a representative gymnosome pteropod, and it is the genus favored by neurobiologists.