

Evolution of Brooding in Sea Anemones: Patterns, Structures, and Taxonomy

DISSERTATION

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By

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Abstract

Brooding is an unusual reproductive behavior among Actinarians (sea anemones). Sea anemones commonly free-spawn gametes producing pelagic offspring that develop independently. In brooding, offspring are retained until the juveniles or adult stage. Brooding behaviors are diverse among sea anemones but some of this diversity is obscured by imprecise or inconsistent terminology. Brooding is taxonomically widespread, but most brooding species are found within Actiniidae. The actiniid genus *Epiactis* is particularly notable for its wide geographic distribution and diversity in characters associated with reproduction. Individuals of the Antarctic, hermaphroditic species *E. georgiana* brood offspring on the surface of the adult, whereas anemones in the gonochoric north Pacific species, *E. handi*, hold offspring within the gastrovascular cavity. Additional *Epiactis* species have been described from the Arctic and Atlantic oceans and include species which aren't known to brood. This variation provides an unusual opportunity to investigate the evolutionary patterns in reproductive characters and ramifications of brooding modes, but also calls into question the monophyly of the genus. This uncertainty is manifested in the literature through taxonomic actions including the creation of *Cnidopus* for several *Epiactis* species and in dispute regarding the specific identity of certain *Epiactis* specimens.

Here I employ morphological and molecular techniques to investigate taxonomic and systematic questions about the genus *Epiactis*, and to investigate patterns in the evolution of brooding in sea anemones. I address taxonomic issues by employing anatomical methods such as histological sectioning, tissue squash preparations and dissection. I make extensive use of museum materials and examine type specimens and other museum lots of the focal species, and collect new specimens from across the known range in order to identify geographic and taxonomic boundaries. I use DNA sequence data to assess relationships among species and investigate the evolutionary patterns in brooding modes and other reproductive characteristics.

Chapter 1 introduces the group and biological phenomena of interest. In chapter 2 the phenomenon of brooding is reviewed broadly across Actiniaria. Terminology used in discussion of brooding is defined. *Epiactis fecunda* is treated in chapter 3 and is reassigned to the genus *Urticina* based on morphological examination of new specimens. In chapter 4, new collections and museum specimens determined to be *Epiactis ritteri* and *Epiactis japonica* are examined. Their specific distinctness is justified with morphological characters, as is the distinctness of the internally brooding individuals from lower latitudes previously identified as *E. ritteri*. The latter individuals are renamed *Epiactis handi*. The final chapter reports on phylogenetic analyses of *Epiactis* species and other actiniids based on nucleotide sequence data. In it, *Epiactis* is revealed to be a polyphyletic group in which North Pacific species and Southern hemisphere species form separate clusters, both with internal and externally brooding representatives. In the North Pacific, external brooding species have evolved from internally brooding ancestors.

This work is dedicated to my unfailingly supportive and understanding wife, Dawn, and to my daughter, Violet, whose intense curiosity and powers of observation never cease to amaze and inspire me.

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- In press* Larson P.G., and M. Daly Phylogenetic Analysis reveals an Evolutionary
Transition from Internal to External brooding in *Epiactis* Verrill (Cnidaria:
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Molecular Phylogenetics and Evolution. <http://dx.doi.org/10.1016/j.ympev>
2015.10.008
- 2015 Larson P.G., and M. Daly ‘Putting Names with Faces’; a description of *Epiactis*
handi sp. nov. helps to resolve taxonomic confusion in species of the sea anemone
Epiactis (Actiniaria, Actiniidae). Journal of the Marine Biological Association of
the United Kingdom 95: 913-928.

- 2012 Larson P.G., J.-F. Hamel, and A. Mercier. Redescription and notes on the reproductive biology of the sea anemone *Urticina fecunda* (Verrill, 1899), comb. nov. (Cnidaria: Actiniaria: Actiniidae). *Zootaxa* 3523: 69–79.
- 2006 DaCosta, M., P. Larson, J.P. Donahue, and S. Weller Phylogeny of Milkweed Tussocks (Arctiidae: Arctiinae: Phaegopterini) and Its Implications for Evolution of Ultrasound Communication. *Annals of the Entomological Society of America* 99: 723-742.

Fields of Study

Major Field: Evolution, Ecology and Organismal Biology

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Chapter 1: Introduction to Sea Anemones, Brooding, and *Epiactis*

Sea anemones (Cnidaria, Anthozoa, Hexacorallia, Actiniaria,) are marine invertebrates that lack hard parts and have a primarily sessile existence as adults. Their bodies are supported by internal water pressure, maintained by bands of densely ciliated tissue which bring water into the animal from the mouth. As cnidarians, they possess microscopic capsules called cnidae which, among other things, can sting and subdue prey organisms by delivering a dose of venom. The descriptions of anatomy, life history, and reproduction that follow are not meant to be exhaustive but are a generalized overview that focuses on the structures, and the relationships of such structures, that are relevant to the reproductive phenomena investigated by this work, and aim to establish a norm for actinarians, against which the unusual features and behaviors under investigation in this work can be contrasted.

General anatomy

Sea anemone bodies are built of a trilaminate sheet, which is folded, evaginated, invaginated, and protruded to produce the structures of the body. The three layers of the body wall are endoderm, mesoglea, and ectoderm, from inner- to outermost, respectively. Endo- and ectoderm are cellular epithelial layers while mesoglea consists largely of proteinaceous and collagenous material and relatively few cells (Figure 1). That sea anemones are essentially folded 2-dimensional organisms has implications for unusual

size scaling in terms of surface area to body-tissue volume ratios and the formation of more complex (i.e. '3-dimensional') structures. Regions of sea anemones' tissues become specialized for certain functions (e.g. gamete production or mucus secretion), but do not form true organs.

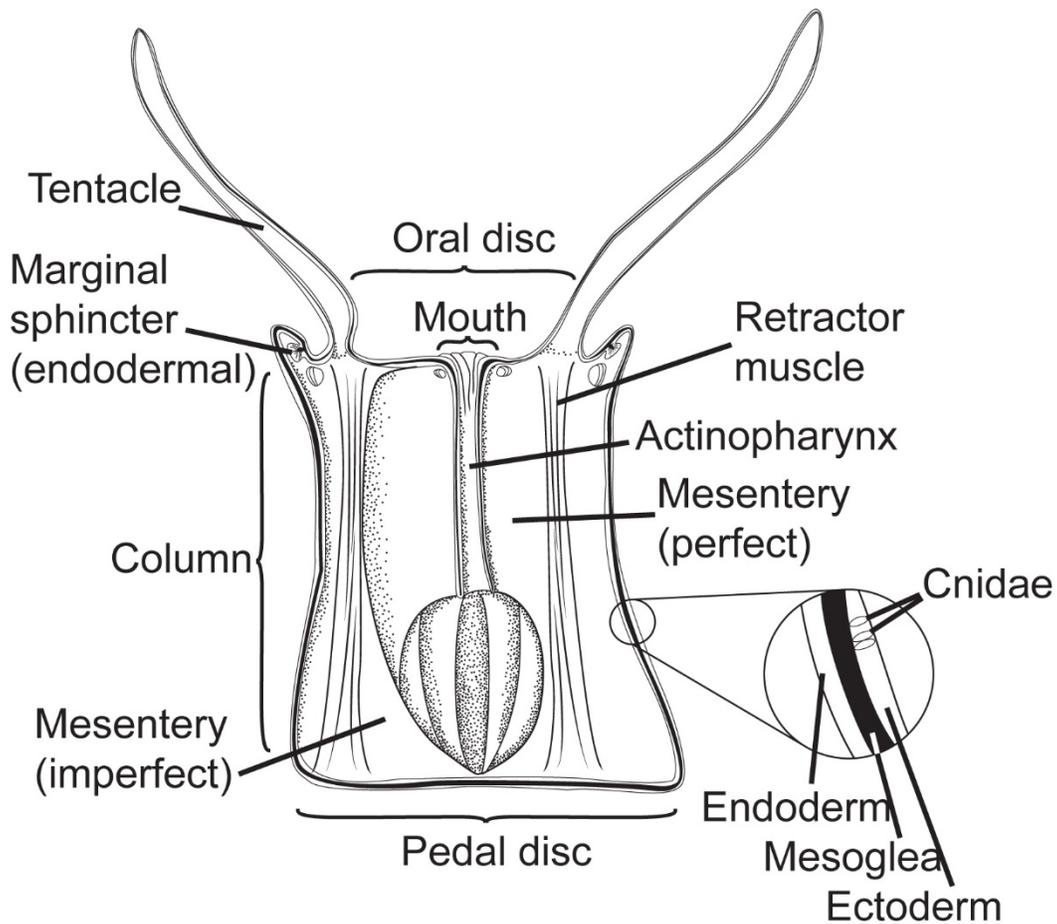


Figure 1 Longitudinal section of an endomyarian sea anemone with major structures labeled.

The general sea anemone polyp body plan (Figure 1) is of a hollow cylinder, the column, internally partitioned by radially arranged walls, called mesenteries, which are attached to the inner surfaces of the cylinder. “Perfect” or “complete” mesenteries connect with the central, tubular actinopharynx which extends towards (but does not connect to) the base of the animal from the mouth. The function of basal or proximal end of the animal is to attach to (via a pedal disc -Figure 1), or burrow into (via a physa -not figured) substrate while the distal end, primarily specialized for feeding, is an oral disc perforated with a central mouth surrounded by cyclically arranged tentacles. Food intake is only one of several functions that the mouth serves. Other functions are expulsion of solid waste, entrance and/or exit for gametes and offspring (although these may also leave through other pores in the body such as those at the tips of tentacles or cinclides of the column), and intake of sea water during expansion of the animal. The tentacles are hollow, and the space within them is continuous with the space within the rest of the body. Collectively this space is referred to as the gastrovascular cavity. Retractor muscles attached along the length of the mesentery allow the animal to contract longitudinally, while the marginal sphincter cinches the top of the column closed, concealing the oral disc and tentacles. Such muscles are produced by tight, repeated folding of the endoderm and mesoglea.

Life History and Reproduction

Whereas the life cycle of many cnidarians includes a benthic polyp stage and a pelagic medusa stage, medusae are always absent from sea anemone life histories. Pelagic dispersal then is up to the planula larva. Planktotrophic pelagic larvae are most common,

but pelagic lecithotrophs are known as well. Commonly, after free swimming for some time, planulae settle upon suitable substrate and metamorphose into juvenile sea anemones, resembling miniature versions of the adult. Juvenile anemones are able to begin using the cnidae in their tentacles to capture and feed upon food items. If conditions permit, the juvenile sea anemone grows, eventually becoming fertile and able to start the life cycle once again.

Reproduction in sea anemones can be coarsely divided into asexual and sexual modes.

While some species are fully capable of prodigious reproduction both sexually and asexually, others are known for sexual reproduction only. Sea anemones as a whole exhibit an array of asexual reproductive tactics, but most species are only capable of one or two of these modes, if any. Regenerative modes of asexual reproduction include such behaviors as pedal laceration, longitudinal fission, transverse fission, and budding. In pedal laceration, fragments of the column and pedal disc of the adult are broken off as the animal moves along its substrate. These fragments then begin to regenerate missing body structures, eventually becoming very small but complete individuals. In longitudinal fission, the animal pulls itself apart as opposite sides of the pedal disc begin moving in different directions. The internal arrangement of anatomy can be somewhat irregular for a time after the two descendent individuals have healed, but internal reorganization can also occur prior to fission. Transverse fission initiates with one or several restrictions at points along the length of the column followed by the separation at the restrictions. The resulting fragments regenerate any missing structures such as oral discs and tentacles, and live independently thereafter. Budding behavior is characterized by the outgrowth of a

new individual from the tissues of another larger individual, and differs from pedal laceration in that the descendent produced is fully formed before separation from the parent. Besides regenerative modes of asexual reproduction, there is some evidence which indicates at least a few species are capable of apomictic parthenogenesis, but since that work is so taxonomically restricted, it is difficult to say how important this mode of reproduction is for sea anemones in general.

Sexual reproduction is widespread but varied among sea anemones. Gonadal tissues develop on the mesenteries. Sea anemones may be gonochoric or exhibit various forms of sequential or simultaneous hermaphroditism. A simultaneous hermaphroditic individual may have mesenteries that individually produce only male or female gametes, or the mesentery itself may be hermaphroditic. Mature gametes are commonly spawned into the surrounding water where fertilization and larval development takes place. In other species, fertilization may be internal, and offspring may not be released from the parent until the larval stage or later.

Brooding

Brooding is a general term for reproductive behaviors that span metazoan phyla as distantly related as Porifera (e.g. a brooding sponge *Rhopaloeides odorabile*) and Chordata (e.g. the Surinam toad *Pipa pipa*). The scope of behaviors covered by usage of the term may differ among researchers in different taxonomic groups, but at least some level of parental care or protection is always implied, which is assumed to result in increased survival of offspring, and is often supposed to carry some potential tradeoff cost, either to the parent, offspring, or both. For example, a sessile brooding species

which bypasses a motile larval stage may show decreased dispersal potential, resulting in direct competition for local resources with one's own offspring.

Brooding in sea anemones is a relatively rare phenomenon, and is characterized by the retention of offspring at least to the juvenile stage. The offspring may be internally brooded within the gastrovascular cavity (Figure 2 D, offspring not visible) or externally, upon the external surface of the adult (Figure 2 A-B, arrows) and may be produced by sexual or asexual means. The genus *Epiactis* is unusual among sea anemones for its high proportion of brooding species.

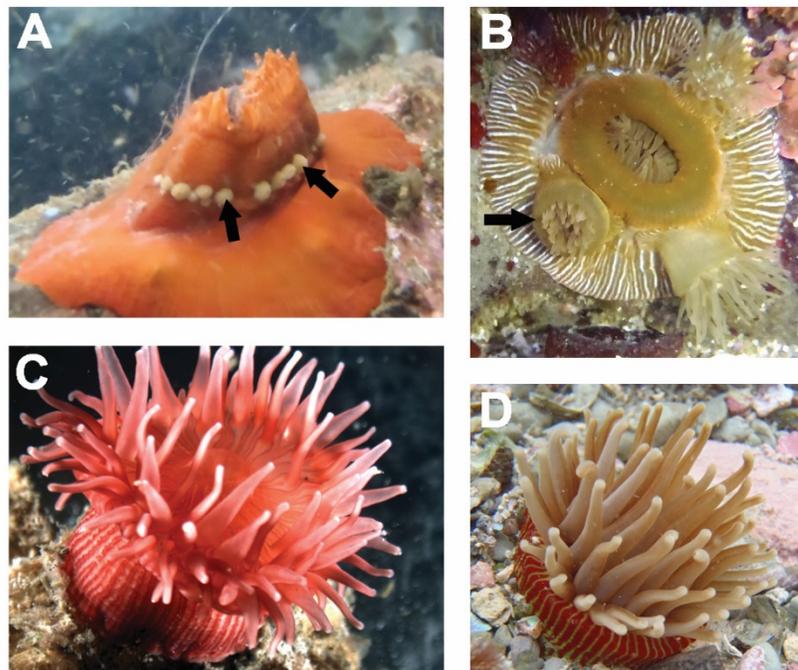


Figure 2 *Epiactis* species in situ. A. *E. ritteri* B. *E. prolifera* C. *E. lisbethae* D. *E. thompsoni*. Some externally brooded offspring indicated with arrow (A. and B).

Chapter 2: A Review of Brooding in Actiniaria

Introduction

Cnidarian reproduction is extremely diverse and well characterized in a broad sense. Among representatives of the phylum, reproductive behaviors include life histories with alternate generations; capability for asexual reproduction via many mechanisms; hermaphroditism and/or gonochory; and various levels of parental care from none at all (free spawning planktotrophic offspring) to extended (protecting offspring through metamorphosis and beyond). The reproductive diversity among sea anemones (reviewed by Bocharova & Kozevich 2011) is nearly equal to that on display in the phylum as a whole. Whereas reproduction in Cnidaria, broadly, and Actiniaria in particular has been reviewed recently, (Fautin 1992, 2002; Bocharova & Kozevich 2011) the following is a more focused treatment of brooding in particular, as exhibited in the Actiniaria -a topic which was last treated comprehensively by Carlgren (1901). Carlgren listed twelve internally and eight externally brooding species. Subsequently, the number of species in which evidence for brooding has been reported has increased to 65 (Table 1). Furthermore, some questions posed by Carlgren have since been answered. The progressively revealed diversity in manifestations of brooding behavior by different researchers in taxonomically disparate groups has led to inconsistent, confusing, or

imprecise use of terminology with respect to brooding itself and behaviors or conditions associated with brooding. This state of affairs makes comparing the works of different authors (and sometimes different works by the same author) difficult. With over a century's worth of research to consider, a distillation of current knowledge is timely. I seek to; 1. Synthesize and organize the current state of knowledge on the brooding phenomenon in actinarians 2. Stabilize terminology pertaining to brooding and brooding behaviors 3. Identify gaps in current knowledge of the brooding phenomenon for future efforts.

“Brooding” refers to diverse and distinct behaviors associated with reproduction across metazoan taxa (a cursory literature search returns brooding species for 17 of 32 searched phyla). A general uniting feature of the various forms of brooding, however, is some degree of parental care of developing offspring in the form of a close physical relationship: usually errant or independent offspring are kept with an adult during development (e.g., mouth-brooding fish), or perhaps an otherwise mobile adult remains with its deposited offspring (e.g., nesting birds) for extended periods. In the extreme contrast to brooding, gametes are freely released from parents and the entire life cycle of the offspring is independent. The apparent continuum in levels of parental care between the two extremes of freely spawned gametes and the extended postnatal care of live-born juveniles suggests that any proposed categories subdividing parental care across animal taxa would be arbitrary. However, the behaviors of parental care may be more discrete within lower taxonomic levels (e.g. Class or Order), but also incomparable between them,

and therefore it is appropriate to propose such categories or distinctions at a restricted taxonomic level, independently of those in other taxa.

In considering three stages of the sea anemone life history (larva, juvenile, and adult), I adopt Chia's (1974, p. 122) definition of a larva as "a developmental stage, occupying the period from post-embryonic stage to metamorphosis, and [differing] from the adult in morphology, nutrition, or habitat." The actiniarian planula larvae differ morphologically from subsequent life stages in that they are simple ovoid in shape, lacking the structures that characterize a polyp such as tentacles, column, and adherent or burrowing base. The early life stages of parasitic forms of sea anemones can be more complex and include alternation between a vermiform parasitic phase which occurs during parasitization of a host and a planula phase which occurs during transition between hosts, and after leaving the final host prior to settlement (Reitzel et al. 2009). Larvae are most often pelagic dispersers, but "demersal" larvae have also been reported (e.g, Riemann-Zürneck 1976a). The juvenile stage has no consistent biological definition but is always characterized by pre-fertility. I define it here as the life stage where pre-fertile individuals have at least one cycle of tentacles and mesenteries, a mouth, and a physa or pedal disk; –in short, they are small and infertile versions of adults. Like larvae, juveniles may be free living, or live in association with (in or on) an adult conspecific. Juveniles may be capable of using tentacles for feeding. An adult is a polyp (usually larger than a juvenile) that is capable of producing gametes. It too may be free living or, more rarely, associated with another adult.

I define brooding for actinarians as the retention of individual offspring by the adult to at least the juvenile stage. While offspring of various species might spend time with or within their parent from zygote through adult, this definition is intended to carry biological and ecological significance rather than be an arbitrarily chosen point with respect to offspring's life stage. In particular, larvae are primarily life stages of dispersal (Chia 1974, but see Riemann-Zürneck 1976a) whereas juvenile sea anemones (with the exception of some non-brooded parasitic forms) are benthic and relatively sessile. Therefore the difference in dispersal capability between pelagic offspring released as eggs or early embryos and those released as larvae is much less than the difference between either of them and those released as juveniles or as adults. That the offspring must be an "individual" distinguishes brooding from behaviors such as in fission or budding where two polyps are continuous with one another through the connection of body tissues, or the continuity of the epithelia in particular.

This definition of brooding is highly consistent with the most common way the term has been used in the literature on Actiniaria, and should result in minimal confusion or outright conflict among authors. Such confusion arises primarily when larviparity (the release of offspring at the larval stage) is referred to as brooding (e.g. Siebert and Spaulding 1976, Mercier et al. 2011). The definition proposed here differs only slightly from Dunn's (1975a) definition: "the retention of offspring by a parent through the embryonic stages usually passed in the plankton, thereby shortening or entirely eliminating the dispersal stage." Note that Dunn (1975b, 1977a) distinguished "juveniles" or "young" from adults by their being located on the adult or living freely, respectively,

rather than by state of developmental maturity. Dunn's scheme has the advantage of being readily applied to individuals in the field but is somewhat arbitrary with respect to development (e.g., a juvenile might graduate into adulthood by simply being forcibly pried from the adult brooding it) whereas the scheme proposed here retains the traditional biological meanings of the life-stage terminology, although adults and juveniles may be indistinguishable at a glance (hence "...at least the juvenile stage."). In short, some of Dunn's "juveniles" are actually sexually mature individuals (1975b), and some of my "adults" may yet be brooded by another adult. I prefer the terms "brooded" and "independent" in exact correspondence with Dunn's "juveniles" and "adults," respectively (i.e., brooded individuals are attached to or held within the adult and independent individuals are attached to some other substrate, irrespective of ontogenetic stage). Some other differences from our terminology include Chia's (e.g. 1976) and Stephenson's (e.g. 1935), use of "viviparity" for what I call "internal brooding," and Sanamyan & Sanamyan's (2006) reference to internally brooded "larvae" with numerous tentacles (=juveniles).

The above definition of brooding is not perfectly free from arbitrariness; asexually produced offspring present a special challenge. For example, species of *Bolocerooides* shed tentacles into their gastrovascular cavities, and these tentacles then regenerate missing structures to become full, miniature sea anemones, which are then released from the gastrovascular cavity to an independent life (Pearse 2002), fitting the conditions of being internally brooded as presently defined. In some instances, however, the tentacles may generate a full polyp without becoming anatomically detached from the adult's

body, in which case they have been considered to be budded offspring (Chia 1976, Shick 1991). The biological and ecological differences between the two cases seem trivial and their distinction may be only technical.

Brooding among Taxa

Brooding species are represented in thirty genera across ten actiniarian families (Table 1). The fact that the largest actiniarian family, Actiniidae (>200 species), contains the most brooding species is unsurprising, but the relative dearth of brooding species in families like Edwardsiidae (100 species), Hormathiidae (110 species), and Sagartiidae (85 species) is notable (species numbers from Daly et al. 2007). The relatively low number of brooding species in these families may be the result of errors of omission: brooding is a seasonal phenomenon for some species and there may be no evidence for the behavior in specimens collected outside of the brooding species (Larson & Daly 2015). It may be that many described species exhibit unobserved brooding behaviors. The large number of brooding species for Actiniidae is partly explained by several large genera with many species (suggesting a common origin of brooding), rather than many independent evolutionary events. Based on current knowledge, however, the minimum number of times in which brooding independently evolved in Actiniidae (eight, according to Larson & Daly in press) is greater than the largest possible number of events in the other large families (e.g., six, for Hormathiidae, if each brooding species represents an independent derivation of brooding).

Family	Genus	Mode	Structure	sex allocation	source
Actiniidae	<i>Actinia bermudensis</i>	internal	---	---	Walton 1918
	<i>Actinia ebhayiensis</i>	internal	---	---	Schama et al. 2012
	<i>Actinia equina</i>	internal	---	Gonochoric+	Carter & Miles 1989
	<i>Actinia prasina</i>	internal	---	---	
	<i>Actinia Sali</i>	internal	---	---	Schama et al 2005
	<i>Actinia tenebrosa</i>	internal	---	---	Farquhar 1898
	<i>Anthopleura atodai</i>	internal	---	hermaphroditic	Yanagi & Daly 2004
	<i>Anthopleura handi</i>	internal	---	Gonochoric	Dunn 1978
	<i>Anthopleura hermaphroditica</i>	internal	---	---	England 1987
	<i>Anthopleura aureoradiata</i>	internal	---	---	Carlgren 1950
	<i>Aulactinia incubans</i>	internal	---	Hermaphroditic	Dunn et al. 1980
	<i>Aulactinia stella</i>	internal	---	hermaphroditic	Verrill 1922
	<i>Aulactinia sulcata</i>	external	chambers	Gonochoric	Clubb 1902
	<i>Aulactinia vancouverensis</i>	internal	---	Gonochoric	Sanamyan & Sanamyan 2013
	<i>Bolocera kerguelensis</i>	internal	---	---	Dunn 1983
	<i>Bunodactis bunodiformis</i>	internal	---	---	Carlgren 1941
	<i>Bunodactis chrysobathys</i>	internal	---	---	Chia 1976
	<i>Bunodactis mortenseni</i>	external	groove	Gonochoric	Carlgren 1924
	<i>Bunodactis octoradiata</i>	external	chambers	---	Clubb 1908
	<i>Bunodactis spetsbergensis</i>	external	groove	---	Carlgren 1921
<i>Bunodactis verrucosa</i>	internal	---	---	Stephenson 1929	

Table 1 Brooding sea anemones. Sex allocation fields with '+' indicate occasional presence of alternate mode and any field containing '---' indicates unreported/non applicable.

Continued

Table 1 Continued

	<i>Condylactis aurantiaca</i>	internal	---	Gonochoric	Schmidt, 1972b
	<i>Cribrinopsis albopunctata</i>	internal	---	---	Sanamyan & Sanamyan 2006
	<i>Epiactis arctica</i>	external	pits	Gonochoric	Verrill 1899
	<i>Epiactis fernaldi</i>	internal	---	hermaphroditic	Fautin & Chia 1986
	<i>Epiactis georgiana</i>	external	groove	Gonochoric +	Dunn 1983
	<i>Epiactis handi</i>	internal	---	Gonochoric	Larson & Daly 2015
	<i>Epiactis japonica</i>	external	groove	hermaphroditic	Uchida 1934
	<i>Epiactis lewisi</i>	external	pitted groove	Gonochoric	Carlgren 1940
	<i>Epiactis lisbethae</i>	external	---	Gonochoric	Fautin & Chia 1986
	<i>Epiactis marsupialis</i>	external	pits	---	Carlgren 1893, 1901
	<i>Epiactis prolifera</i>	external	---	Hermaphroditic+	Dunn 1977a
	<i>Epiactis ritteri</i>	external	groove	Gonochoric	Larson & Daly 2015
	<i>Epiactis thompsoni</i>	internal	---	---	Stuckey 1909
	<i>Isoaulactinia stelloides</i>	internal	---	hermaphroditic	belem et al. 1996
	<i>Oulactis muscosa</i>	internal	internal pits?	Gonochoric	Stuckey 1909
	<i>Parantheopsis cruentata</i>	internal	internal pits?	gonochoric +	Kirk & Stuckey 1909
	<i>Parantheopsis georgiana</i>	external	chambers	Both	Carlgren 1899
	<i>Parantheopsis vanhoffeni</i>	external	chambers	Hermaphroditic	
	<i>Urticina crassicornis</i>	internal	---	---	Carlgren 1921
	<i>Urticina fecunda</i>	external	pits	Gonochoric	Larson et al. 2012
Actinostolidae	<i>Actinostola callosa</i>	internal	---	---	Carlgren 1901

Table 1 continued

	<i>Antholoba achates</i>	internal	---	hermaphroditic +	Fautin 1984
	<i>Glandulactis spetsbergensis</i>	internal	---	Gonochoric	Riemann-Zurneck 1976b
	<i>Hormosoma scotti</i>	internal	---	gonochoric	Carlgren 1927
	<i>Stomphia coccinea</i>	internal	---	hermaphroditic	MacGinitie 1955
	<i>Stomphia selaginella</i>	internal	---	Gonochoric	Fautin 1984
Aiptasiidae	<i>Aiptasia mutabilis</i>	internal	---	---	Stephenson 1929
Boloceroiidae	<i>Bolocerooides "sp. B"</i>	internal	---	---	Pearse 2002
	<i>Bunodeopsis medusoides</i>	internal	---	---	Pearse 2002
	<i>Bunodeopsis sp.</i>	internal	---	---	Pearse 2002
Edwardsiidae	<i>Edwardsia timida</i>	internal	---	---	Rawlinson 1935
	<i>Edwardsia vivipara</i>	internal	---	---	Carlgren 1950
Halcampidae	<i>Halianthella annularis</i>	external	groove	---	Carlgren 1938
	<i>Halianthella kerguelensis</i>	external	chambers	---	Carlgren 1901
Hormathiidae	<i>Calliactis parasitica</i>	internal	---	---	Stephenson 1935
	<i>Cataphellia brodricii</i>	internal	---	---	Stephenson 1935
	<i>Cricophorus nutrix</i>	external	groove	---	Stuckey 1908
	<i>Hormathia coronata</i>	internal	---	---	Stephenson 1929
	<i>Hormathia digitata</i>	internal	---	---	Stephenson 1935
	<i>Hormathia incubans</i>	internal	---	---	Carlgren 1934b
Isanthidae	<i>Isanthus capensis</i>	internal	---	---	Carlgren 1938
Phymanthidae	<i>Epicystis crucifer</i>	internal	---	Gonochoric	Jennison, 1981
Sagartiidae	<i>Cereus pedunculatus</i>	internal	---	---	Stephenson 1929
	<i>Sagartia troglodytes</i>	internal	---	Hermaphroditic	Stephenson 1929

Brooding Modes

Brooding in sea anemones can be subdivided into two modes. Externally brooded offspring remain in contact with the adult's ectoderm whereas internally brooded offspring remain within the gastrovascular cavity. "Internal" and "External" modes are thus delimited by which side of the adult's body wall the offspring contact as juveniles rather than where they physically occupy space: externally brooded offspring may actually take up some volume of the gastrovascular cavity by means of occupying deeply invaginated "chambers" in the adult's column that impinge on the coelenteron (see section on brooding structures below; Figures 3-5). Internally brooded offspring and chamber-dwelling offspring may share similar levels of protection by the parent and comparably low exposure to the environment, but these qualities are not a discrete way of categorizing brooding modes since externally brooded offspring may be exposed fully, as in *Epiactis prolifera*, enclosed entirely by the parental tissues (at least for part of their development), as in *Epiactis Ritteri*, or something to an intermediate degree (e.g., *Epiactis marsupialis*). Defining external and internal as is done above is discrete and also captures a difference in behavioral complexity: internal brooding can be effected simply through a delayed release of internally fertilized offspring, whereas external brooding requires a release of offspring but the addition of some mechanism or behavior to retain them upon the adult. These are intended as categorical groupings and do not imply or preclude homology of the behavior among species exhibiting the same mode.

Internal brooding

Internal brooding is simply the development of offspring within the gastrovascular cavity of an adult. It is an inconspicuous behavior compared with external brooding, yet many more internally brooding species have been identified (Table 1). Internal brooding may simply be effected by the delayed release of offspring (i.e., by extending oviparity and/or larviparity resulting in a viviparous condition) by the maternal parent, yet there is some evidence for more complex life histories in certain taxa.

Production and brooding of offspring

By far, the most well studied internally brooding species belong to the genus *Actinia*. A flurry of research activity through the 1970s and 1980s was initiated by Chia & Rostron (1970) who reasserted Gravier's (1916) proposal that that in *Actinia equina*, offspring were released from the adult for some developmental period, then returned to gastrovascular cavity of adults as larvae to be brooded through the juvenile stage. This proposal was meant to explain the observation that males, females, and nonsexual individuals all contain offspring in a given population (Chia & Rostron 1970). While it has been shown that *A. equina* will successfully foster the introduced offspring of other individuals under experimental conditions (Lubbock & Allbut 1981), numerous behavioral and genetic studies have indicated that *A. equina* adults will not voluntarily ingest larvae or juveniles placed on or near their mouths, and brooded offspring of *A. equina* are actually produced asexually (reviewed by Jennison 1981, Perrin et al. 1999) via somatic embryogenesis. The importance of sexual reproduction is still unknown in

many brooding species of *Actinia*, although in *A. bermudensis*, larvae produced sexually are not brooded (Jennison 1983). In contrast, in their study of the internally brooding actiniid *Aulactinia stella*, Bocharova & Mogue (2012), reported genetic evidence that at least some sexually produced offspring are naturally brooded by adults other than a possible parent, but it is unknown how offspring move from one adult to another and how long they spend in transit. In *Phymanthus crucifer*, male individuals internally brood juveniles (Jennison 1981). Jennison (1981) preferred a sex-change hypothesis to account for the pattern in *P. crucifer*. The phenomenon of the brooding male is apparently more common than previously expected, as it is also reported by Sanamyan & Sanamyan (2006) in *Cribrinopsis albopunctata*.

The other known asexual mode of reproduction in internal brooders exists in the Boloceroididae. In species of *Bunodeopsis* and *Boloceroides*, tentacles autotomized while retracted within the gastrovascular cavity can regenerate the rest of the body to form full polyps (Cutress 1979, Pearse 2002). In *Bunodeopsis medusoides*, the offspring are formed through regeneration at the base of the tentacle (in which distal end of the founding tentacle forms one of the new polyp's tentacles), or in the middle of the tentacle (in which the proximal and distal end of the founding tentacle of the autotomized tentacle form opposing tentacles of the new polyp (Pearse 2002). Given the penchant for asexual reproduction in these species (including common and uncommon modes such as oral scission, longitudinal fission, and pedal scission, the fact that most tentacle fragments develop within the gastrovascular cavity may be incidental rather than an instance of adaptive brooding.

That offspring of gonochoric species are produced sexually might be a tempting assumption but the above examples demonstrate an important caveat. *Actinia equina* is gonochoric, yet the offspring found within it are produced asexually. Given the diversity among taxa, modes of reproduction in any actinarian species should be confirmed genetically before the commencement of any study that relies upon that information. Nonetheless, seasonal coordination of reproduction in a gonochoric species is strong evidence for the sexual production of offspring.

Dynamics

The number of internally brooded offspring at any given time is variable between species and probably within species. An individual of *Epiactis handi* contained only one offspring (pers. obs.) but up to 85 have been reported from a single individual of *Cataphellia brodricii* (Stephenson 1929).

Different developmental stages of offspring may be localized within the adult.

Actinostola spetsbergensis broods enigmatic “giant larvae,” spherical propagules that approach 1cm in diameter. Riemann-Zürneck (1976a) reports that the developmental stages spanning zygotes through juveniles at the 24-mesentery stage are brooded in the distal portion of the adult, and contained individually within an “embryonal envelope[s].” At the same time, mature stages, including those with tentacles (24 of which appear simultaneously) are held within the gastrovascular cavity near the base of the adult, apparently having “hatched” from the envelope.

Larval interactions in the larviparous species *Urticina felina* could explain some phenomena observed in internally brooding species. Offspring of *U. felina* are known for chimerism via the fusing of two or more embryos (Mercier et al. 2011). The release of fused larvae may result in multiple settled and metamorphosed polyps joined together by tissues (morphological aberrants), or of single (“homogenous”) polyps composed of multiple larval sources (Mercier et al. 2011). Fusion could explain the size of larvae within *Actinostola spetsbergensis*, and the occurrence of offspring with multiple oral disks and tentacular crowns in the broods of *Epiactis thompsoni* (pers. obs.).

External brooding

External brooding is the first brooding mode to be reported. Verrill (1868) thought the pre-juvenile offspring of *Epiactis arctica* were eggs of a parasite, a mistake he corrected upon description of the externally brooding *Epiactis prolifera* (Verrill 1869). External brooding requires that offspring must leave the gastrovascular cavity of the adult and become attached to the outer surface. Superficially, it appears to be a more complex behavior that offers less protection than internal brooding. The behavioral details of externally brooding species are variable, however, and some externally brooded offspring are nearly as concealed from the environment as internally brooded individuals are, -at least part of their development.

Production of offspring

Externally brooded offspring are the product of sexual reproduction in the few species in which it has been investigated (Ishimura & Nishihira 2002, Bucklin et al. 1984, Dunn 1975b). *Epiactis prolifera* is a gynodioecious hermaphrodite (Dunn 1975a). Ova about 400 microns in diameter are produced by *E. prolifera* (Dunn 1975b); these are negatively buoyant, as is expected to be necessary to keep eggs near the parent in externally brooding species (Dunn 1975b, Larson & Daly 2015). Offspring are produced continually (mixed sizes of offspring) among members of these populations.

Individuals above 7.5mm may maintain as many as 35 individuals of mixed size (Dunn 1977a), further evidence of continuous reproduction (Dunn 1975b). Brood size is necessarily related to body size of the parent. Relatively larger species of *Epiactis* and its relatives (i.e., *E. japonica*, *E. ritteri*, *U. fecunda* (Larson & Daly 2015, Larson et al 2012) may hold hundreds of juveniles, generally fairly uniform in size, at least at the beginning of their seasonal reproductive cycle, with the number decreasing and/or the diversity of sizes increasing over the reproductive season.

Brooding structures

Externally brooded offspring are held, in the simplest case, upon the surface of the column of the parent and are thus hypothesized to be vulnerable to environmental elements such as dislodging due to wave action, ultraviolet light exposure, desiccation, and direct predation. The degree of protection may depend on the position of the adult. For example, when an adult of *E. prolifera* is expanded, its tentacles typically hang over

the offspring, presumably protecting them to some extent from above. When the adult retracts, however, offspring are as exposed as they would be attached directly to the substrate (but see discussion of Dunn 1977a and Fautin & Chia 1986 below). In other species, specializations of the parent's column may help mitigate the risks of exposure by containing and covering offspring to varying extents. These features are varied in form and may be permanent or temporary. Temporary modifications can disappear as the offspring grow upon the adult, or may remain for the full duration of the offspring's tenancy, disappearing only after the offspring have left the adult.

The terminology used to refer to such structures in sea anemones is inconsistent among authors, and even within the body of a single author's work. For example, Carlgren (1949) referred to all brooding structures as "brood pouches" except those in *Cricophorus nutrix* which he called "brood rooms." But in 1924, he referred to those of *C. nutrix* as both "pouches" (e.g. p. 258) and "rooms," (e.g. p. 257), and he referred to dissimilar brooding structures in *Epiactis marsupialis* as both "pits" and "rooms." The word "chamber" also refers to these structures in general (see Bocharova & Kozevich 2011). While it appears the terms "pouch," "room," and "chamber" are meant to be synonymous, the inconsistency is confusing and obscures the differences in structures among species. The casual mention of "pouches" in species accounts (e.g. Carlgren 1927) is not informative unless an explicit anatomical description of the pouch or a clear illustration is provided. Based on anatomical study and a synthesis of literature accounts, I suggest more restricted and specific application of these terms. These structures can be divided into three major categories: pit, groove, and chamber.

Pits

A brooding pit is an invagination in the column which contains a single developing offspring (Figure 3). Pits are shallow but include multiple layers of the body wall to some degree. Verrill (1868) first noted pits in *Epiactis arctica*, where individual embryos develop within shallow invaginations of the column. The pits involve at least the ectoderm and mesoglea, form by a thinning of the mesoglea (Verrill 1899), are open, with occupants partially exposed, and are arranged in a regular quincunx pattern near the base of the adult. For *E. arctica*, Carlgren called them “pits,” (1921) and “pouches” (1949); similar structures in *Epiactis marsupialis* were called “Höhlungen” (Carlgren 1893) and “rooms” (Carlgren 1921). Offspring of *Urticina fecunda* develop within individual pits on the column of the parent; adjacent and very closely set pits remain separated by a thin projection of the body wall in *U. fecunda* (Larson et al. 2012). While structures matching the above description of “pit” have been called by other names, the term “pit” has rarely been applied to other types of structure. Furthermore, “pit” was Verrill’s (1899) original term. Thus I favor “pit” as the term explicitly referring to this type of structure.

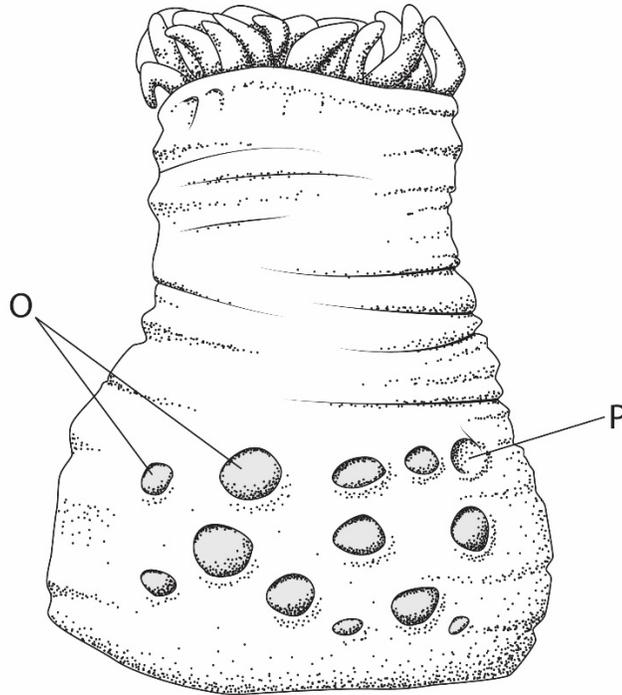


Figure 3 Brooding pits in the column of *Epiactis arctica*. O-offspring, P-empty pit.

The one taxon in which the specific term "pit" has been applied in an ambiguous way is *E. prolifera*. Verrill (1899: p. 378) referred to the slight thinning and depression of the body wall beneath externally brooded offspring as pits, but noted that they “never become like the deep pits of [*E. arctica*, and *U. fecunda*].” In *E. prolifera*, even the earliest stages of brooded offspring are held on the surface of the adult’s column rather than in circular depressions; forcible removal of live juveniles from an adult *E. prolifera* reveals a discolored patch but no clear pit-like depression. In fixed specimens, a shallow depression like a “footprint” may be left behind when offspring are removed. Pits as defined here are therefore absent in *E. prolifera*.

Grooves

A brood groove is an annular or semi-annular depression in the column formed by folding of the body wall (Figure 4). In *Epiactis japonica* and *Epiactis Ritteri*, the grooves are not permanent features of adult morphology, but are present for some portion during the beginning of the brooding period (Carlgren 1924, Ishimura & Nishihira 2002, Larson & Daly 2015). Uchida & Iwata (2002) propose a function of egg retention for the brood groove in *E. japonica*: members of this species are often attached to non-horizontal surfaces and release pre-planular (i.e. non-motile) offspring. Groove formation in *E. japonica* has been described by Ishimura & Nishihira (2002): a normally flattened anemone contorts itself into a dome shape; it bends its column, producing a fold; the mouth protrudes in the direction of the bend; zygotes or early embryos are released through the mouth directly into the fold. During deposition, the mouth moves in a circle depositing offspring as it revolves around the animal. The groove may partially or fully cover the offspring at the time of deposition (Ishimura & Nishihira (2002), but later in the brooding season, the groove disappears and the juveniles are exposed upon the surface of the adult (pers. obs.). In the related species, *Epiactis Ritteri*, the brood groove is further specialized in that the column tissues above and below the groove meet and become tightly sealed together, fully enclosing the offspring (See chapter 4, Larson & Daly 2015). Dunn (1983) described a groove-like structure in *Epiactis georgiana* that forms near the distal portion of the adult's column and in which offspring are brooded. The collar of the adult folds down to cover the offspring, and this region of the column has

“glandular patches” which may aid in attachment of juveniles (Dunn 1983). Carlgren (1924) described an “annular invagination” in *Cricophorus nutrix* (Figure 4) and a “deep fold” surrounding the column and hiding some offspring in *Epiactis mortenseni*, but noted that “the embryos had mostly immigrated from the [groove].”

Dunn (1977a) described an ephemeral brood groove that may cover smaller juveniles when the adult is contracted in *E. prolifera*, and a similar structure has been described for the seasonal external brooder *Epiactis lisbethae* (see Fautin & Chia 1986). I have not seen this groove in populations of *E. prolifera* that I have studied; rather, contracted adults appeared smoothly dome-shaped and juveniles on their surfaces were plainly visible. In *E. prolifera*, a permanent groove like the one described for *E. japonica* may be impossible because *E. prolifera* reproduces on a semi-continual basis, with a single adult concurrently hosting a wide size range of juveniles. This explanation would not apply to *Epiactis lisbethae*, which is a seasonal brooder (Fautin & Chia 1986). Features of this kind may serve an important protective function especially when the tentacles of the adult are retracted, but since they seem to be an artifact of the animal’s immediate posture rather than a long-term formation of the column, I consider *E. prolifera* and *E. lisbethae* to have unmodified columns with respect to the brooding structures discussed here.

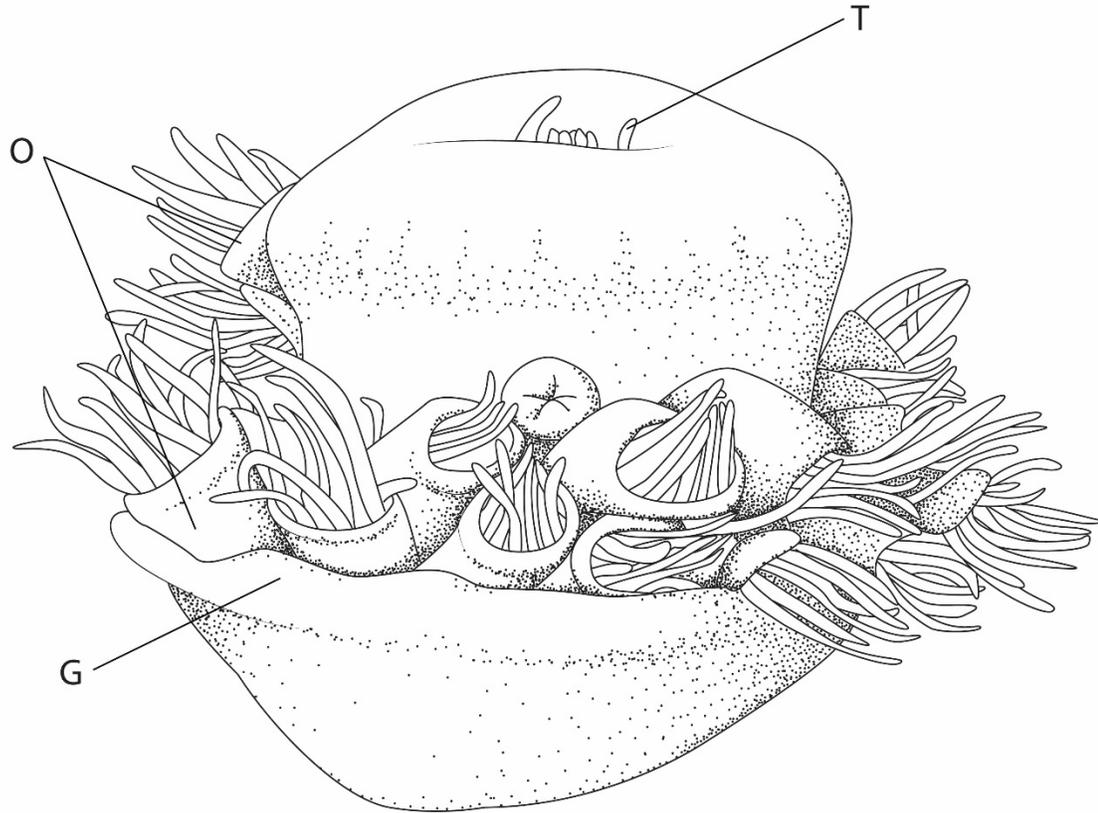


Figure 4 Brood groove in *Cricophorus nutrix*. O-offspring, T-tentacle of adult, G-outer rim of groove.

Chambers

Brood chambers (Figure 5) are the most uncommon and possibly most complex brooding structures documented for actinarians. Chambers are characterized by a deep invagination in the column that expands internally and holds multiple offspring. The pouches of *Halianthella kerguelensis* serve as a model for this type: six narrow apertures

around the animal lead to large cavities which each contain many (>100 for some chambers) developing offspring (Carlgren 1893, 1901).

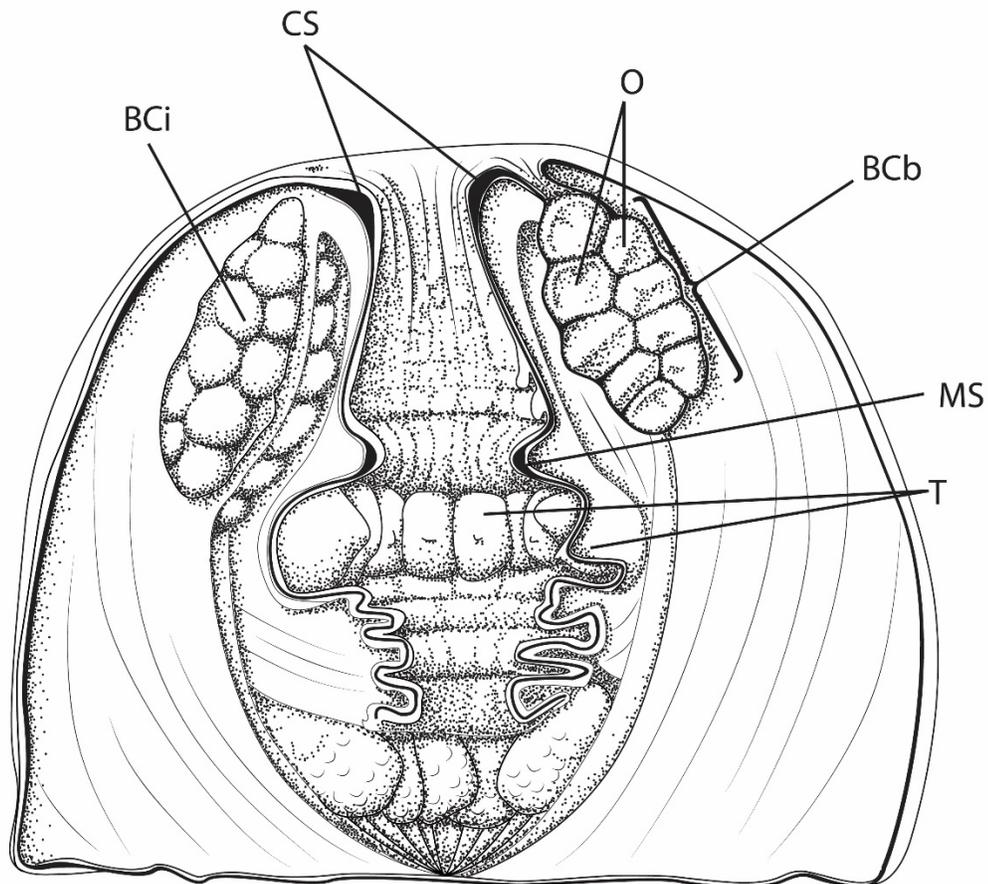


Figure 5 Brood chambers of *Halianthella Kerguelensis*. BCI-intact brood chamber, BCb-bisected brood chamber, CS-column sphincter, MS-marginal sphincter, O-offspring, T-tentacle. After Carlgren 1901, Figure 12.

Parantheopsis georgiana also contains chambers which may hold up to three offspring but these are distributed across the surface of the column rather than in a ring around it

(Carlgren 1899, 1927). *Aulactinia sulcata* also appears to have something like chambers, but the anatomical description and formation of these structures are disputed (See below). The structures described above might form in combination with one another. In *Epiactis lewisi*, offspring develop in ‘pockets’ at the base of deep circular invagination, likely the fosse, based on Carlgren (1940, Figure 2b). In total, this structure might resemble a groove lined with pits, although grooves are typically on the column and this appears to form entirely within the perimeter of the marginal sphincter. In the *E. lewisi* specimens I have examined (CAZ 025092), I have seen a very shallow (but empty) groove just proximal to the sphincter on the column in most specimens, but nothing medial to the sphincter as is described and depicted by Carlgren (1940).

Uncertain or dubious structures

Enigmatic structures of uncertain affinity with those listed above have been described. Stuckey (1909) identified “brood-pouches” in the endodermal surface of the oral disk for internally brooded ova in *Oulactis muscosa*. It is not stated to what developmental state these offspring are held, and brooding and pouches have not been mentioned in subsequent reports of *O. muscosa* (e.g., Parry 1951, Marshall et al. 2004). *Aulactinia sulcata* is described by Clubb (1902, Figs 18, +19, Pl. LII) as having “brood chambers” marked by a “constriction” or “groove” running around the animal; this appears to be a single continuous cavity formed by an annular depression, consistent with “groove” as is defined here. The text, however, describes a means of formation quite unlike that described by Uchida and Iwata (2002) for *E. japonica*: deep pits form and then expand to

connect with one another, resulting in four to six larger depressions. He inferred that the mesenteries between the pits break down in the process. Carlgren & Stephenson (1929) disagree, noting that the brooding spaces are all fully continuous with one another, formed by a single invagination of the column which forks inside the animal and forms an incomplete ring. Dunn (1983) reported a single empty invagination, wider internally than at its aperture in *A. sulcata*. In any case, this structure appears to be most similar to a chamber as presently defined, but descriptions of new collections would help confirm this.

A feature common in externally brooding taxa that may serve a protective function are holotrichous isorhizae, or holotrichs. Nematocysts do not contribute to the physical envelopment of the offspring, but holotrichs at and near the limbus of the adult may be defensive against some benthic predators that approach from the substrate. Holotrichs have been reported, at least sparsely, from the columns of all externally brooding species of *Epiactis* in the North Pacific. In *E. ritteri* and *E. japonica* however, they are numerous at the broadly flared base, which forms a flat band around the animal and nearly doubles its basal diameter (Larson & Daly 2015). In addition, holotrichs are found in the most marginal tentacles of these same *Epiactis* species; these tentacles are the ones that are on the outside when the adult expands and covers the young with its tentacles. Holotrichs are also linked with the evolutionary transition between internal and external brooding in *Epiactis handi* (See Larson & Daly in press). Extrapolation from other cnidarians suggests that such a defensive perimeter may be effective: holotrichs of hydroids cause tissue damage to predatory nudibranchs (Martin & Walther 2002). On the other hand,

aeolid nudibranchs consume *Epiactis prolifera* (see Waters 1973), although the column of this species is relatively sparsely populated with holotrichous nematocysts (when they are present at all) compared with *E. ritteri* and *E. japonica*. It is unknown to what extent nudibranchs are able to prey upon the latter species. Holotrichs also feature in intraspecific aggression (e.g., Purcell 1977) and the flared base may be a way of preventing the encroachment of conspecifics, though I have not observed such dense aggregations in the populations I sampled that individuals seemed to be pressured for space.

Formation of structures

The absence of pits, grooves, and chambers in non-brooding individuals of externally brooding species (or their reduced, apparently regressing state) has led some authors to suggest that the structures are induced by the attachment of offspring. In the one species for which formation of a brood groove has been directly observed (Ishimura & Nishihira 2002), it is produced by the actions of the parent at the time of spawning. In the other cases, the process must be inferred by the various states of multiple preserved individuals or simply remain unknown.

Carlgren initially (1893) believed that smaller, empty brood pits in the column of *E. marsupialis* were in the process of formation in anticipation of an occupant, however, upon discovery of females lacking such pits, he decided they must be in regression after the departure of offspring. He concluded that the pits are absent until induced by the presence of offspring and that the pit and young simultaneously grow in size. In *Urticina*

fecunda, the immediate mechanism by which brood pits are formed is unknown, but they do form after the arrival of larvae, and are reduced to absence at some later point (Larson et al. 2012). Whether the pits are formed by the parent for larvae to find and settle in or whether they are locally induced by the presence of larvae is unknown but it is probably a different mechanism than that by which many offspring arrive within the same chamber of, e.g., *Halianthella kerguelensis*. Carlgren (1901) suggests that the brood chambers in that species, due in part to their regular distribution and the multitude of inhabitants of each one, must form prior to the arrival of offspring, although they too grow as the offspring grow.

Offspring deposition

The mechanism by which externally brooded offspring reach their ultimate place of development, whether upon the column or within some specialized structure of the column has been a source of speculation for many years. Contrary to Verrill's (1899) description of eggs being externally brooded, Carlgren (1901) doubted that eggs could actually attach to the body wall of the parent. He instead claimed that ciliated embryos (or larvae) must, in all cases, swim from the gastrovascular cavity to the external surface of the parent where they induce the formation of specialized column structures when such are present. He was equally dismissive of the idea that the adult could, through its own behaviors, place eggs upon itself, though Clubb (1902) supposed that adults used their tentacles to actively place offspring within what must be pre-formed brooding structures.

In several cases, movement of offspring to their place of development has been described in detail. Dunn (1975b) described spawning in *Epiactis prolifera*: early stage offspring (small pink to orange spheres) were ejected from the mouth onto the oral disk; through a heaving action of the oral disk, the spheres were moved to the margin, which they fell over; the offspring initially attached to the column via a mucosal mass. Attachment may be haphazard, with many offspring tumbling to the substrate while others remain on the adult. The fate of unattached pre-juvenile offspring is presumably death, since individuals smaller than 4mm are not found apart from those on adults (Dunn 1977a, pers. obs.) Offspring deposition has not been described in *E. georgiana*, but the appearance of larvae or embryonic stages within the gastrovascular cavity and upon the external surface of adults (Rodriguez et al. 2012) suggest that eggs or zygotes are not spawned, but offspring may rather reach their point of development upon the adult under their own power. The release of larvae and their locomotion to their eventual brood space upon the adult has been noted in *Urticina fecunda* as well (Larson et al. 2012).

Depending on the species, development may be direct or include a larval stage. *Urticina fecunda* and *Epiactis georgiana* are two externally brooding species in which a larval stage is present (Larson et al. 2012, Rodriguez et al. 2012) and is the mode by which offspring move from within the adult to upon it. *Halianthella keguelensis* probably requires a larval stage to account for the large number of offspring within relatively few chambers (Carlgren 1901). Carlgren (1901) assumed that any externally brooded offspring must go through a larval stage to explain the means by which they become

externally brooded, but there are examples to the contrary. In *E. prolifera* and *E. japonica*, the ciliated larval stage is bypassed and development is direct (Uchida & Iwata 1954, Dunn 1975b).

Brooding duration

The time spent in development within or on an adult seems extremely variable across taxa based on reports in the literature, though direct comparison is often impossible because offspring may be described only by size or number of tentacles and mesenteries in preserved specimens rather than actual length of incubation period. There does not appear to be any obvious trend with regard to size of offspring achieved and brooding mode or adult size.

Internal

Phymanthus crucifer is brooded up to the 24 tentacle stage (Jennison 1981), *Urticina crassicornis* can be found within the adult with up to 76 tentacles (Carlgren 1901). According to Pearse (2002), *Bolocerooides* “species B” broods offspring for as little as 4 days and releases juveniles with 8 or 9 tentacles. *Actinia equina* remain with the adult until they are up to 7mm diameter (Chia & Rostron 1970). *Phymactis crucifer* offspring are released at up to 6mm (Jennison 1981). *Antholoba achatas* broods offspring up to 1cm diameter, and approximately 70 tentacles (Dunn 1984).

External

In *Epiactis prolifera*, offspring remain on the adult (or at least do not survive independent of the adult) until they are approximately 4 mm in basal diameter, and most offspring

have left the parent by the time they reach the 4.8 to 6.7 size range (Dunn 1977a). Through repeated measurements at regular intervals, Dunn (1977a) calculated this to mean a brooded period of at least 12 weeks. In *E. japonica*, offspring may infrequently remain on the adult for a period of over 1 year, resulting in the overlap of multiple broods (Shinohara, unpublished data). *Epiactis ritteri* offspring may achieve a relatively large size while remaining on the parent, reaching a pedal disk diameter of up to 16.5mm (Larson & Daly 2015).

Nutrition

Given the positive correlation between prey size and pedal disk diameter found in some species (Shick 1991), brooded offspring and adults are unlikely to be in competition for the same food resources. The problem of feeding in offspring who have exhausted their yolk supply may be quite different among the brooding modes though. Externally brooded offspring whose tentacles are exposed to the environment for at least some portion of the time should be able to catch some of their own food. Predation has been confirmed in the brooded offspring of *E. prolifera* and *E. ritteri* based on the contents of the gastrovascular cavity of offspring (Dunn 1975b, Larson & Daly 2015). Although externally brooded offspring would be able to acquire energy through photosymbiosis in theory, such a mode of nutrition is unknown for any of the species that brood externally. The external source of energy for offspring brooded within pouches or deep pits is presently unknown, if any exists; it may be that offspring leave such structures before or shortly after the yolk has been depleted. However, actinarians can use dissolved organic matter (DOM) (Shick 1991) as a source of nutrition and this may be important for

juveniles bathed in sea water but not exposed to significant sources of prey. Although Verrill (1869) suggested internally brooded offspring derive nutriment from the adult, no known instance of matrotrophy has been documented.

There is some evidence that internally brooded offspring may feed directly on food items ingested by parent (Chia & Rostron 1970). Internally brooded 12- and 24- tentacle stage individuals of *Phymanthus crucifer* were capable of feeding on *Artemia* in the lab immediately upon removal from the parent (Jennison 1981). Utilization of DOM (Shick 1991) within the adult's gastrovascular cavity is also a possibility, albeit unmeasured thus far.

Release and dispersal

Because adults are generally sessile and benthic, larval dispersal is important to the ecology of many sea anemone species. Few studies explicitly measure distances traveled by pelagic larvae, but long range dispersal is inferred from conformation to Hardy-Weinberg predictions and low FST values across large ranges. For example, *Bunodosoma caissarum* larvae are long lived and populations in Brazil show little substructure (FST = 0.042) across 1150 km (Russo et al. 1994). Brooding species in *Epiactis*, on the other hand, show higher levels of population subdivision with FST values ranging from 0.157 in *Epiactis handi* to 0.364 in *Epiactis lisbethae* across ~1300 km (Edmands and Potts 1997).

Externally brooded offspring can become independent by simply crawling off the adult. Ricketts et al. (1964) describe *E. prolifera* offspring as "gliding" away. Internally brooded

offspring may be ejected from the adult through mouth as in *Actinia equina*, or through terminal pores in tentacles, as in *Aulactinia incubans* (Dunn et al. 1980). Offspring of some species may also be ejected, probably prematurely, upon disturbance of the adult. Shortly after collection of adult specimens of *Epiactis thompsoni*, many tiny individuals could be found in the bags and buckets holding the adults (pers. obs.).

Although pelagic dispersal is the best-known strategy for anemones, not all actiniarian larvae are pelagic (Reimann-Zurneck 1976a), and the bypassing of a pelagic dispersal stage means that long range dispersal, if it occurs, must be restricted to other means. The following modes of transportation are not restricted to brooding species only, but brooding species are restricted to these.

Crawling

Sea anemones may move from one location to another across the substrate by several modes. Dunn (1977b) described an inch-worm like movement of alternating detachment, progression, and attachment of the leading and trailing edges of the pedal disk in her studies of movement in *E. prolifera* in the field. Movement was largely undirected (except during a predator encounter) and the net change in position was often smaller than the gross distance traveled (Dunn 1977b); the greatest net travel recorded was 66mm over 30 days. *Actinia tenebrosa* in the field are somewhat more active, traveling up to at least 7.5 cm over the course of 16 days (Ottaway & Thomas 1971). Some species employ an additional modes of benthic locomotion. Species of *Bolocerooides* can detach the pedal disk from the substrate, attach tentacles to the substrate and then contract, release other tentacles, systematically pulling themselves along a distance of several body diameters in

the oral direction (Josephson & March 1966). It is unlikely that these modes of crawling across the benthos by formerly brooded individuals account for much dispersal, and Dunn (1977a) noted circumstantial evidence indicating offspring simply settle very near their parents.

Swimming

Short, relatively fast relocations may be effected by swimming. Swimming events may only incidentally contribute to dispersal and are presumed to be primarily escape responses to predators because they are initiated by contact with other organisms such as sea stars of the genus *Dermasterias* and nudibranchs (Dalby et al. 1988). By lashing their tentacles in unison, species of *Bolocerooides* swim at speeds nearing 2cm/second and for durations up to 182 seconds (Josephson & March 1966). *Stomphia coccinea* and *Stomphia didemon* (reported by MacGinitie (1955) as an internal brooder) are other swimming species that move short distances by releasing their pedal disk and rhythmically bending the column back and forth.

Phoresy

Calliactis parasitica, a “fairly frequent” internal brooder (Stephenson 1935 p. 239) is known for being often transported by hermit crabs which actively attach the sea anemones to their shells (Wortley 1863, Fox 1965). This species is also oviparous, however, and dispersal via crab is of uncertain significance compared with that of the pelagic larval stage.

Rafting

Probably the most significant mechanism for long range dispersal and the founding of new populations is rafting. Rafting is the movement of otherwise sedentary organisms on floating objects from one locality to another (Hobday 2000). It may only occur in sporadic events, but can facilitate rapid dispersal over long distances. Bryan et al. (2012) report a rafting event in which at least 5000 km were covered in less than a year. Sea anemones have been found rafting on pumice (Bryan et al. 2012) and plastic (Goldstein et al. 2014). Brooding species such as *E. prolifera* can be found attached to potential rafts items such as *Ulva* and *Zostera* sp. (Dunn 1977b), and have been found persisting on kelp rafts that are older than 50 days (Hobday 2000). Likewise, in the southern hemisphere, *Cricophorus nutrix* is commonly found on *Lessonia* sp. (Stuckey 1909) and on *Marginariella* sp. (pers. obs.), an alga with pneumatocysts.

Brooding functions, costs, and benefits

Brooding, in general, is often assumed to increase the fitness of the adult through the enhanced survivorship of offspring at some cost to the parent. There is some evidence from sea anemones and other taxa that the assumption that brooding increases offspring survivorship is a valid one. Ottaway (1979b) noted that the size of offspring released affects chance of survival. Brooding allows more offspring to reach a large size before experiencing competition for space and other resources, and being exposed to predators or other agents. Menge (1975) has found that brooding increases the probability of individual offspring survivorship to sexual maturity in *Leptasterias* sea stars over

broadcasting in *Pisaster ochraceous*. In brooding sea anemones, offspring of *Epiactis prolifera* apparently do not survive at all unless they are brooded (Dunn 1975a).

While almost no quantitative studies have explicitly investigated the costs or benefits of brooding in sea anemones, the lack of dispersal stage suggests that the common dispersal related tradeoffs may be important in these species. In brooded species, juveniles are already in a suitable habitat for settlement, potential mates are likely nearby, and offspring have bypassed a very dangerous life stage. On the other hand, reduced dispersal increases the potential for inbreeding, environmental fluctuations can threaten one's entire brood, and adults may eventually compete for resources with their own kin. In this context, brooding may serve to mitigate reduced fitness caused by inbreeding depression. For example, as a gynodioecious and brooding species, *E. prolifera* is subject to potentially high levels of inbreeding through self-fertilization and through fertilizing offspring who have settled nearby. Population genetic studies of this species (Bucklin et al. 1984) have shown low genetic diversity at the population level and evidence for self-fertilization in this species. Pilakouta et al. (2015) have experimentally demonstrated in *Nicrophorus* beetles that individuals produced through inbreeding receive higher fitness benefits from parental care than outbred individuals do. Furthermore, since detrimental effects of inbreeding can be exacerbated by environmental stressors (Fox & Reed 2011), brood protection may be especially important in these species.

Certain *Actinia* species appear to have circumvented some of these problems. *A. bermudensis* and *A. equina*, for example, spawn gametes, producing sexual offspring

which disperse from the parent, thereby deriving the potential benefits of recombination and dispersal. They also internally brood asexually produced offspring, thereby gaining the benefits of protection and suitable settlement site. Furthermore, because the coefficient of relationship (r) between brooded individuals and the adult is 1, there is no competition between the adults and these nearby offspring.

Ecological and geospatial patterns

For much of the 20th century, nonpelagic development in general and brooding in particular was considered to be a cold-water phenomenon. The idea that pelagic development is absent from polar and deep waters was promoted by Gunnar Thorson (1950) and was called Thorson's Rule by Mileikovsky (1971). The crux of the argument in support of Thorson's rule is that the brevity of the phytoplankton bloom increases with latitude and therefore lecithotrophy becomes more important, and pelagy is therefore a needless risk. Brooding was thought to be a response to lack of food for larvae and low temperatures.

The downfall of Thorson's Rule is well documented in reviews on the topic (Pearse 1994, Young, 1994, Pearse & Lockhart 2004), which highlight that brooding in marine invertebrates is currently understood to be a mostly Antarctic phenomenon rather than a general cold water one, and pelagic and planktotrophic development are now known to be common in many species in cold waters.

As the historically accepted geographical patterns of non-pelagic development and brooding began to break down with the arrival of new, and the revisitation of old data, so

did the need for ecological explanations to explain those patterns. In brooding Antarctic species, a lineage diversification explanation (powered by rafting events and dispersal by the Antarctic Circumpolar Current) suffices to account for the abundance of closely related brooding species rather than adaptation to Antarctic conditions per se (Pearse et al. 2009).

There are no clear geographic patterns in the distribution of brooding sea anemone species in general, or of internally brooding species in particular. The behavior is nearly pan-latitudinal and pan-longitudinal (Figure 6). Externally brooding species, on the other hand, appear to be absent from the tropics (Figure 6). This distribution was remarked upon by Clubb (1902) and continues to be evident after over a century of additional collecting. It is unlikely to be an artifact of incomplete knowledge because far more species have been described from the low and mid latitudes than from the polar seas (Fautin et al. 2013). In addition, external brooders represent several distinct lineages rather than a radiation of a single group (Table 1). These geographic and systematic patterns in external brooding suggest that some ecological factors may yet favor external brooding under certain conditions, or simply discourage it under others. The large swaths of ocean without brooding species (Figure 6) are mostly deep sea habitats which are relatively poorly known in any case. It may yet be that internal brooding is primarily a shallow water phenomenon, but that is not evident from the data at hand, and some species that brood internally are found in several-thousand meter depths (e.g. *Hormathia coronata*, >5000m,) (IFREMER, 2015).

Any hypothesis proposed to explain geographic patterns of brooding based on abiotic conditions such as climate (e.g., Highsmith 1985) is inapplicable to sea anemones because there is no clear pattern (in the general case). Therefore a specific mode and its particulars must be dealt with independently. Clubb (1902) noted the geographic pattern and suggested that species which brood externally may have a longer development than would be “convenient” in the gastrovascular cavity. Long development time is a characteristic of cold water development (Hoegh-Guldberg & Pearse 1995), but the presence of internal and external brooders in cold water raises doubt for that explanation. Since there is no clear distribution pattern for internal brooders, internal brooding may simply be a fitness-equivalent alternative to oviparity, larviparity, and (in some environments) external brooding. External brooding, on the other hand, may be an adaptation to current or recent conditions in the ranges where it occurs. The bimodal high latitude distribution tempts explanations which invoke adaptations to high latitude life. On the other hand, external brooding may confer no distinct advantage or disadvantage in these environments, but simply be maladaptive in the tropics.

One hypothesis is that high predation pressure in the tropics may favor internal brooding (great protection but few offspring) and free spawning (no protection but many offspring) over external brooding (few offspring and only moderate protection). Although Ates’ (1989) list of fish known to prey upon sea anemones includes observations spanning from Greenland to New Zealand and includes internally brooding prey species (*Actinia equina* and *Sagartia troglodytes*), brooding mode seems unlikely to be important to offspring survivorship when relatively larger fish species such as *Pleuronectes* or *Gadus* may eat

the entire animal (Ates 1989). Rather, smaller predators which are only capable of taking small bites of the adult or removing individual offspring would seem more likely to be the major factor causing differential survivorship of offspring between brooding modes. While aeolid nudibranchs have been observed feeding on *E. prolifera* (Dunn 1977b, Hall & Todd 1984) the importance of nudibranchs as predators to any one sea anemone species is uncertain because Aeolids may be conditioned to prefer certain sea anemone species (Hall & Todd) and populations may have differing regional preferences (Waters 1973).

Correlates of brooding in other taxa

Micromorphism

Brooding in marine invertebrates is often noted to be associated with small adult size. The pattern is generally restricted to comparisons among closely related groups in which the smaller species tend to be brooders rather than a trend in absolute body size among all invertebrates (Strathmann & Strathmann 1982). Hypotheses proposed to explain this phenomenon often involve tradeoffs in sexual reproduction between brooding and nonbrooding in small vs. large individuals. Menge (1975) suggests that brooding in *Leptasterias hexactis* is an adaptive strategy that compensates for a small adult size (and reduced fecundity) induced by interspecific competition with *Pisaster ochraceous*. Other explanations (reviewed by Strathmann & Strathmann 1982) compare capacity for gamete production with brood space at various sizes, longevity with size and optimal reproductive strategy, or dispersal dynamics associated with size. Kalfuss et al. (2013) found no statistically significant difference in size between mantle-cavity brooding

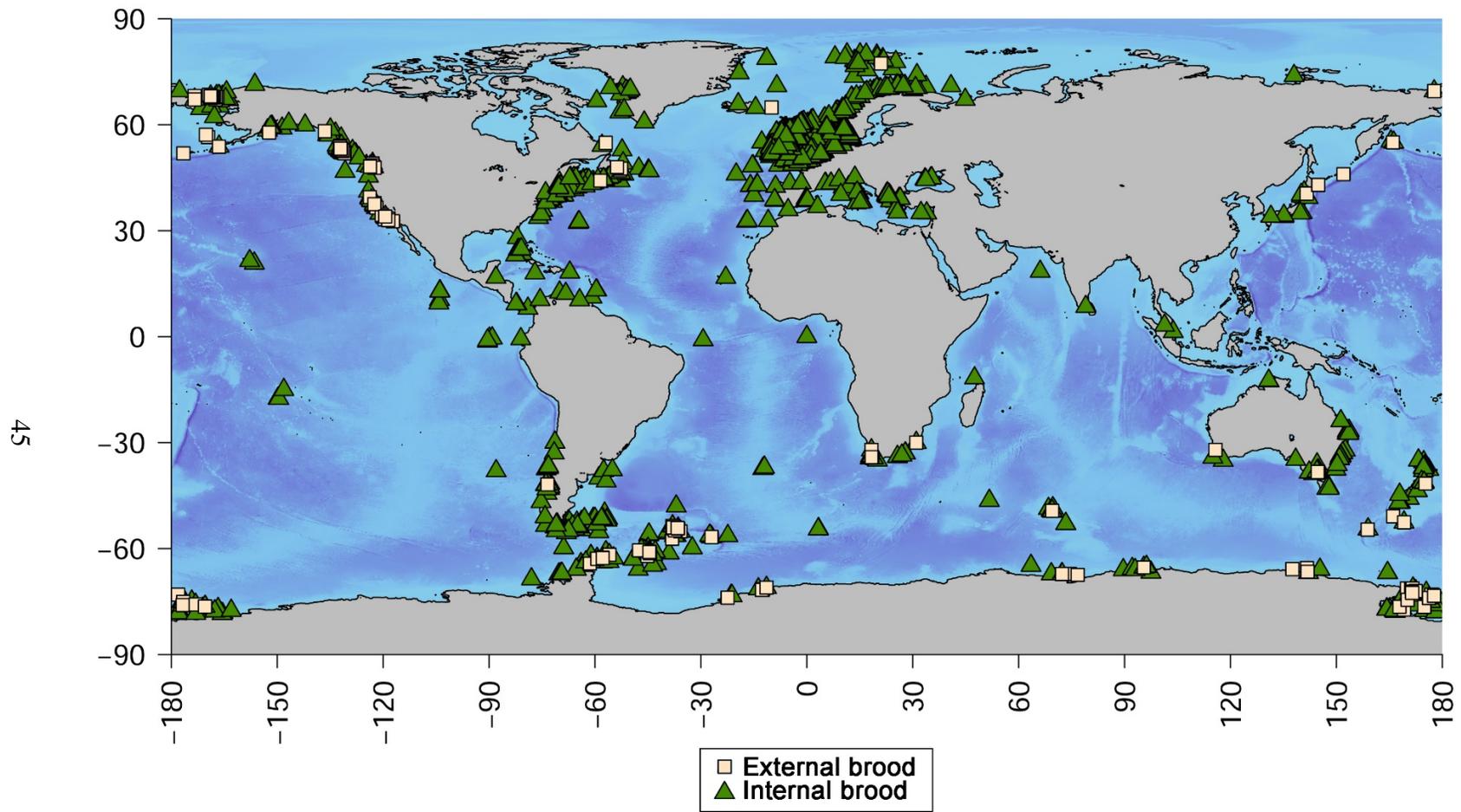


Figure 6 Locality data for species listed in table 1. Data from Fautin 2015 or Global Biodiversity Information Facility (GBIF)

brachiopods and non-brooding species, but those which brood in specialized pouches were significantly smaller than mantle brooders and non-brooders.

Whether sea anemones demonstrate this correlation, and whether proposed explanations are applicable to sea anemones is unclear based on available data. In addition, sea anemones do not necessarily stop growing at adulthood, may shrink under unfavorable conditions, and their laminar construction complicates the inferences that can be made from size and growth with respect to gamete production potential (Shick 1991).

Importantly, smaller sea anemones may not be geometrically similar to larger ones, and therefore the properties of isometry cannot be applied to estimate gamete production potential based on, e.g., pedal disk diameter. For example, a growing individual may simply undergo roughly isometric growth for some time, with its mesenteries (the site of gamete production) expanding in size and gamete production potential increasing at a predictable rate. At some point, though, the animal could also begin adding another cycle of mesenteries, which may or may not become fertile, depending on the species, and which may or may not be predictable in number. This mode of growth adds anatomical complexity to the animal and potentially new gametogenic tissue sites that will now begin expanding in the manner of the previous cycles. Any study seeking to test hypotheses about size and brooding in a context of gamete production could only be successful in a well quantified system.

Ignoring the complex issues of growth, size and brooding do not seem to have a clear relationship in the species currently known to brood. Within *Epiactis*, relatively larger (*E.*

ritteri) and smaller (*E. prolifera*) species both brood. In addition, the very few putative *Epiactis* species that are not known to brood are small, but described from only one or a few specimens (e.g., *E. irregularis* 1.1cm, Carlgren 1951; *E. vincentina*, 2 cm, Carlgren 1939) and an accurate size range of those species is unobtainable. Brooding species *Urticina fecunda* and *U. crassicornis* are larger than *Epiactis* species, and neither are considered dwarfed with respect to their congeners. Among *Actinia* species, the relatively large *A. striata* (6 cm pedal disk diameter) broods offspring internally while smaller *A. schmidtii* (3.7cm pedal disk diameter) does not brood (Perrin et al. 1999).

Hermaphroditism

Another classic correlate of brooding is hermaphroditism (Ghiselin 1969). Kaulfuss et al. (2013) and Strathmann et al. (1984) proposed or tested explanations uniting hermaphroditism, small size, and brooding in specific taxonomic cases. For brachiopods, it was proposed that brood-protecting within special pouches and simultaneous hermaphroditism were compensatory responses to the small adult size and short life span of those species (Kaulfuss et al. 2013). Strathmann et al. (1984) studied brooding sea stars to test Heath's (1977) hypothesis that larger individuals might produce more eggs than can be successfully brooded (caused by allometry of growth in gonad and brood space), and therefore should stay small and allocate excess reproductive resources to sperm production. Strathmann et al. (1984) found some evidence for allometry, but not for reallocation of efforts to spermatogenesis. Instead they suggest that hermaphroditism and self-fertilization are more likely to persist in benthic and brooding species. The

genetic penalty for self-fertilization is relatively reduced in benthic and brooding species because they generally already have high degrees of inbreeding and low genetic diversity at the population level, even in gonochoric populations, due to low dispersal (Strathmann et al. 1984). Furthermore, the consistently low contribution of effort towards male gametes in hermaphrodites of different sizes is consistent with selfing and possibly paired mating but Heath's (1979) hypothesis is therefore inapplicable (Strathmann et al. 1984). The applicability of these hypotheses to sea anemones is uncertain. There is very little data on life span of sea anemones *in situ* and comparisons between brooding and non-brooding taxa have not been made. Genetic diversity has been studied in some species, with the hermaphroditic brooding species *E. prolifera* showing low levels of genetic diversity and evidence of self-fertilization (Bucklin 1984) consistent with the theoretical expectations of these conditions. More importantly, brooding species of sea anemones do not appear to be especially likely to be hermaphroditic. Of the brooding species where sex allocation is known, the majority are gonochoric (Table 1). Larson & Daly (in press) failed to detect a correlation of these characters in *Epiactis*, a genus whose members show diversity of sex allocation and brooding mode. Perhaps because sea anemones only produce gonadogenic tissues rather than true gonadal or other sexual organs, sex allocation is highly evolutionarily labile (Shick 1991).

Predictions from other taxa and Open Questions

Brooding in sea anemones is a diverse phenomenon and an interesting case study that appears to follow, in part, historically accepted but not phylogenetically general

geographical trends (e.g., external brooding being a bipolar phenomenon) but that fails to follow recent and otherwise uncontroversial generalizations (e.g., correlation with hermaphroditism, body size). Satisfactory explanations for these deviations have not been proposed or tested empirically, and could provide a basis for future studies of the evolution of brooding behavior. Current understanding of this phenomenon is meager, and below I identify some knowledge gaps and propose some yet unexplored questions about brooding in Actinarians which may prove to be fruitful avenues of research in the future. I hope that such lines of inquiry may contribute to another review on brooding in sea anemones in, hopefully, less than another 114 years.

How do the costs and benefits of the brooding modes compare with one another?

Empirical studies comparing internal and external brooding in sea anemones are lacking. Internal brooding arguably provides more physical protection to the offspring than does simple external brooding. Furthermore, in external brooding, there is an additional potential for loss of offspring between spawning from the adults mouth and attachment to the column. In addition, since internally brooded offspring occupy a volume within the adult while externally brooded ones require surface area (excepting chambered offspring), internal brooders should keep up with the increasing space demands of their broods as they grow and produce annually increasing numbers of offspring more effectively than external brooders would. What factors then might promote the evolution of external brooding from internally brooded ancestors, as has been shown for in *Epiactis* species in the north Pacific (Larson & Daly in press)?

Internally brooded offspring occupy some volume within the adult that might otherwise be utilized for other biological needs such as ingested food items or production of gonadal tissues whereas externally brooded offspring (in the simple case) do not. Whether this is a true tradeoff is uncertain because the size of internally brooded individuals and their numbers vary on an interspecific and sometimes intraspecific basis (Table 1), and non-brooding species do not necessarily fill their available body volume with prey and gonadal tissues. Offspring brooded externally but within deep chambers also occupy volume within the GVC and more than they would otherwise, since they are surrounded by the invaginated body wall as well. External offspring are not exposed to the adult's digestive enzymes and do not ingest any of food captured by the parent. How brooding changes the behavior of adults is unknown. This may be especially important for nutrition in internally brooding species. Externally brooded offspring (with tentacles) can acquire their own food items from the surrounding water, but anything that internally brooded offspring ingest must first be ingested by the adult, and the adults may increase feeding efforts in response to holding an internal brood. On the other hand, a gastrovascular cavity full of offspring may make feeding more difficult for the adults, and the secretion of digestive enzymes may be dangerous to offspring if these are not well covered by mucous or some other form of protective covering, and alternatively feeding may be inhibited during the brooding period. The presence of offspring might affect other behaviors of the adult including frequency and duration of locomotion, expansion & retraction patterns, likelihood of engaging in intraspecific agonistic competitions, and more, all for equally speculative reasons.

Dynamics of the brood itself is another potentially fertile research area. What proportion of initially spawned externally brooded offspring remain on the adult until they reach a size capable of independent survival -e.g., 4mm pedal disk diameter in *Epiactis prolifera*? In *E. prolifera*, many spawned offspring don't ever become attached to the column, let alone survive through development (Dunn 1977a). Other externally brooding species that spawn and retain many hundreds of eggs or embryos do not have so many later in the season when offspring are in the juvenile stage, and in many cases there is clearly not enough column area to retain the entire brood through its development. In *E. ritteri*, for example, brooded offspring can have a pedal disk diameter of over 1cm (Larson & Daly 2015). In an extreme case, in *A. sulcata*, only 4 to 6 large juveniles are found. Whether these the last individuals of a larger brood, or typical numbers for the species is unknown. Duration of brooding periods are largely unknown for most species. If space and other resources required by offspring are limited, then there must also be competition among broodmates that increases once yolk is exhausted and space becomes scarcer. Only Dunn (1977a) has attempted to quantify offspring attrition rate. Of 350 oocytes found within an average sized adult, perhaps only 10% are successfully brooded on the column and only approximately 3 may survive to reproduce on their own. There are many unknowns among the characteristics of brooding systems in sea anemones. Nonetheless, current knowledge is sufficient to demonstrate that the brooding phenomenon, as it is manifested in these animals, provides a critical check on the generality of conclusions about brooding based on research in other taxonomic groups.

This fact will, hopefully, inspire widespread investigations in under-studied groups and lead to a more complete understanding of the phenomenon across its many incarnations.

Chapter 3: Identity of a putative Atlantic *Epiactis* species

Introduction

Since its original description in 1899 based on two preserved specimens, no subsequent collection of the species currently known as *Epiactis fecunda* (Verrill, 1899) has been reported in the literature, nor have details of its life history nor descriptions of the live animal been given. Originally described as *Epigonactis fecunda*, this species was noted to retain developing juveniles (i.e. to brood its offspring) within individual pits in the distal portion of the external surface of its column. Carlgren (1901) synonymized the species with its only congener, *Epigonactis regularis* (Verrill, 1899) and transferred the species to *Epiactis*, a genus notable among actinarians for its many brooding species.

Although Verrill's original description mentioned and depicted verrucae on the column of his specimens of *Epigonactis*, a feature contrary to any diagnosis of that genus (see Verrill 1869a; Stephenson 1918, 1922; Carlgren 1921, 1949), Carlgren's (1901) recommendation that *Epigonactis* be synonymized with *Epiactis* has been broadly adopted (e.g. Stephenson 1918, 1922; Dube 1974; Sebens 1998). Carlgren (1901) also considered one individual of the type series of *Leiothealia spetsbergensis* (Kwietniewski, 1898) to be *Epigonactis fecunda*. This individual is the source for European records of *E. fecunda* in biodiversity databases (e.g. European Register of Marine Species website), but

the currently confirmed distribution of the species is subtidal deep waters of Nova Scotia and southwestern Newfoundland (see discussion).

In addition to these taxonomic issues, the mode of attachment of offspring to the adult has been of some interest. Verrill (1899) suspected that offspring of externally brooding species attached to the body wall as eggs or zygotes, but Carlgren (1901) thought it more likely that attachment occurred at the motile planula stage. Directly developing eggs or zygotes have been previously reported as being actively placed by the parent (Ishimura and Nishihira 2003) or expelled with mucus (Uchida and Iwata 1954; Dunn 1975b), but until now planulae have not been observed attaching externally to adults (see below).

Here we redescribe the species first called *Epigonactis fecunda* Verrill, 1899 from the type specimens and from new collections from the northwest North Atlantic Ocean. We transfer the species to the genus *Urticina* Ehrenberg, 1834 and report on the seasonal timing of its reproduction, on its brooding habits, and on the early growth of its juveniles. *Urticina fecunda*, comb. nov., is the first described species of *Urticina* known to externally brood offspring.

Methods

Observation of live sea anemones. Seven specimens were collected by SCUBA (see below for geographical coordinates), and associated data on substrate were noted where available. The specimens were transferred to a large tank (600 L) at the Ocean Sciences Centre of Memorial University for monitoring (of gamete release, planulation, brooding) on an opportunity basis during a period of 3 years. The tank contained natural substrates

(mix of boulders, sand, and crushed shells) and was supplied with unfiltered running seawater (15–20 L min⁻¹), at ambient temperature under natural photoperiod and planktonic food supply.

Morphological study of preserved individuals. Cnidae were measured from squash preparations of tissue from column, tentacle, actinopharynx, and mesenterial filament of preserved specimens. Cnidae were measured with a digital video measurement system at 1000x magnification under phase contrast. Up to approximately 40 cnidae of each type encountered were measured following a search pattern described by Williams (1996). No special effort was made to find the extreme size range limits of a given type. Tissues for histological sections were sectioned at 10 µm. Sections were stained using a modified Heidenhain Azan procedure (Presnell and Schreibman 1997). General anatomical observations were made under a dissecting microscope, at maximum magnification of 60x.

DNA barcode sequencing. Samples of sea anemone tissue (from the pedal disk) preserved in 100% ethanol were shipped to the Canadian Centre for DNA Barcoding (CCDB), University of Guelph, Canada, for DNA analysis. Two specimens were analyzed using standard polymerase chain reaction (PCR) and DNA sequencing protocols (Ivanova *et al.* 2006; DeWaard *et al.* 2008). Partial COI sequences with all meta-data are registered in the Barcode of Life Data System, project code SAB97.

Material examined

Type material was acquired from the National Museum of Natural History, Smithsonian Institution (USNM), Yale Peabody Museum (YPM), and Swedish Museum of Natural

History (SMNH). New specimens are deposited at the Canadian Museum of Nature (CMNI).

USNM 24329 *Epigonactis fecunda* syntype; east of Banquereau Bank (Nova Scotia, Canada), 275 meters.

USNM 24885 *Epigonactis regularis* holotype; fishing banks, off Newfoundland, in deep water.

YPN 34734 *Epigonactis fecunda* syntype; Banquereau Bank (Nova Scotia, Canada), 366 meters.

SMNH 5692 *Leiotelia spetsbergensis* syntypes (2 specimens); Spitsbergen, Norway.

Urticina fecunda, comb. nov., Cape Broyle (Newfoundland and Labrador, Canada); 47°05'38" N, 52°55'46" W, 10 meters (1 specimen, new material).

Urticina fecunda, comb. nov., Logy Bay (Newfoundland and Labrador, Canada); 47°37'34" N, 52°39'39" W, 20 meters (1 specimen, new material).

CMNI 2011-0001 *Urticina fecunda*, comb. nov., Island Cove, (Newfoundland and Labrador, Canada); 48°00'36" N, 53°46'02" W, 15 meters (1 specimen, new material).

Reproductive behavior was observed in three specimens before they were preserved (Cape Broyle, Logy Bay, CMNI 2011-0001) and anatomical data were collected from five (USNM24329, USNM 24885, Cape Broyle, Logy Bay, YPN34734).

Complementary and comparative anatomical observations were performed on *Urticina felina* (Logy Bay (Newfoundland and Labrador, Canada); 47°37'34" N, 52°39'39"W, 15-20 meters (2 specimens, new material).

Results

Order Actiniaria Hertwig, 1882

Family Actiniidae Rafinesque, 1815

Genus *Urticina* Ehrenberg, 1834

Urticina fecunda (Verrill, 1899), new combination

Synonymy

Epigonactis fecunda Verrill, 1899: 378

Epigonactis regularis Verrill, 1899: 380

Epiactis fecunda (Carlgren, 1901: 483.–Stephenson, 1922: 274.–Dube, 1974: 33.–Sebens, 1998: 11, 14, 46, 51)

Not *Leiotelia spetsbergensis* (Kwietniewski, 1898: 134,137)

Diagnosis

Urticina with verrucae densely arranged in longitudinal rows. Basitrichs of column occurring in two non-overlapping size ranges. Tentacles 72-92. Mesenteries and tentacles occurring in three cycles, the first being sterile. Other mesenteries may bear large eggs,

approximately 2mm in diameter. Seasonally produced offspring developing to juvenile stage in pits on exterior of column of adult.

Description

Pedal disk: In live animal, pink with lighter, almost white spots, as wide as column and oral disk, ~80 mm. In formalin preserved specimens (retracted), circular, concave, robust, gray. Mesenterial insertions not apparent. Diameter narrower than column, 28–35 mm.

Column: (Figure 7 B, C) In life, cylindrical, longitudinally furrowed near limbus, coloration marbled pink and white. Mid-column and distally, background color solid salmon-orange, with white verrucae (Figure 7 B, C, Figure 8 A) to which stone and shell debris may adhere. Verrucae densely arranged in longitudinal rows, several fusing at parapet. Verrucae less dense proximally, absent nearest limbus (Figure 7B, C). Collar and fossa distinct. During brooding season, distal column bearing several hundred pits (Figure 9) containing offspring of similar or slightly lighter color than column of adult (Figure 7B, D). Oral disk and tentacles completely covered in contracted individual. Acrorhagi and cinclides absent.

Column of formalin-preserved specimens 3.5–6.6 cm diameter; all body tissues creamy off-white or greenish (USNM 24329). Verrucae of preserved specimens centrally dimpled. Despite strong transverse wrinkles, longitudinally furrowed region of proximal column evident.

Oral disk and tentacles: In life, oral disk broad, red, with darker maroon stripes corresponding to insertion of mesenteries (Figure 7A). Not densely crowded with tentacles: 72–92 tentacles in 3 cycles, first cycle decamerous, others may be irregular.

Figure 7. Live individual of *Urticina fecunda*, *comb. nov.* A. Oral disc and tentacles B. Whole individual with adherent debris and externally brooded offspring. C. Non-brooding phase, without pits in column. D. In partial retraction, with brooded offspring, v-verruca. Scale bars = 1 cm.

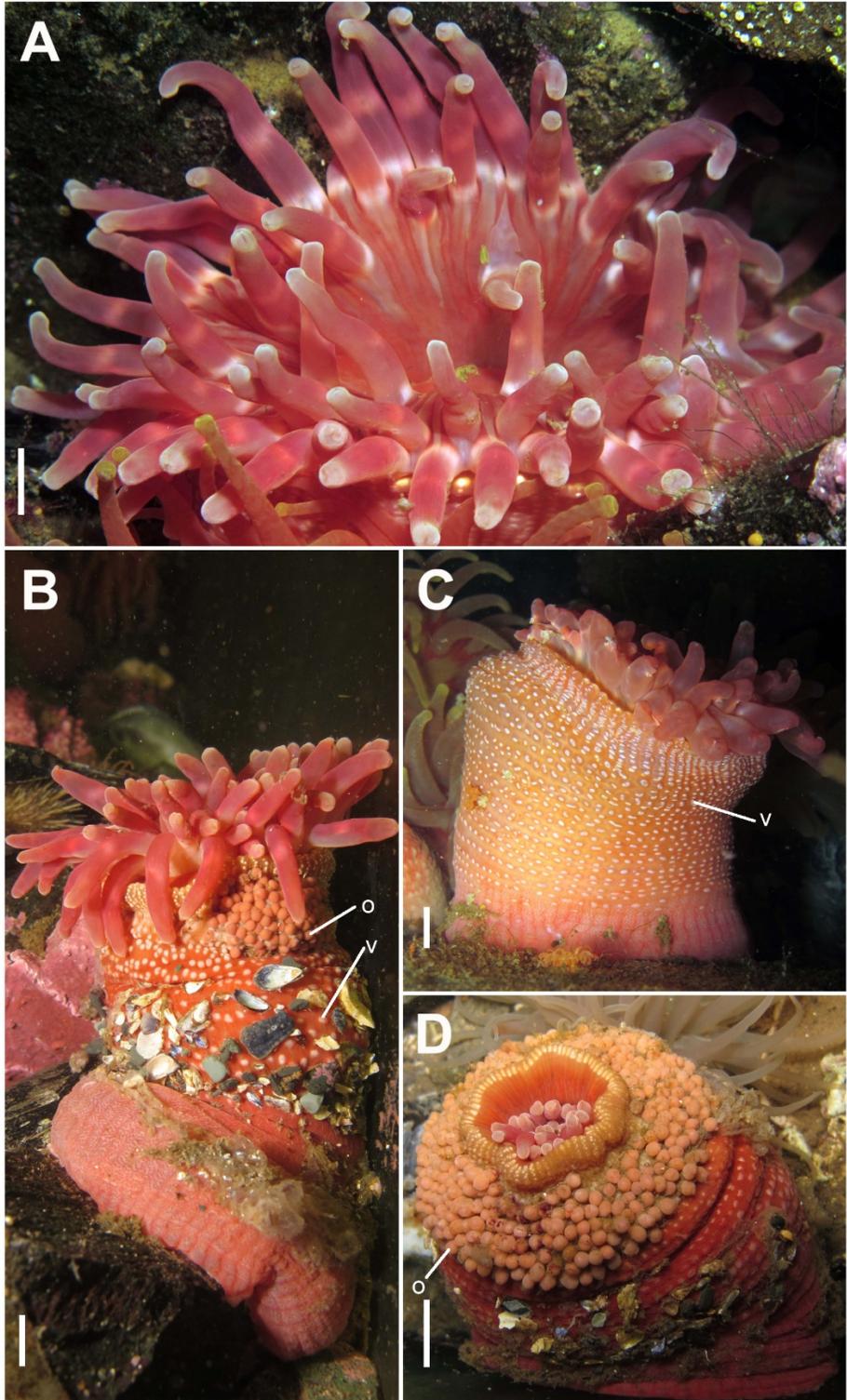


Figure 7
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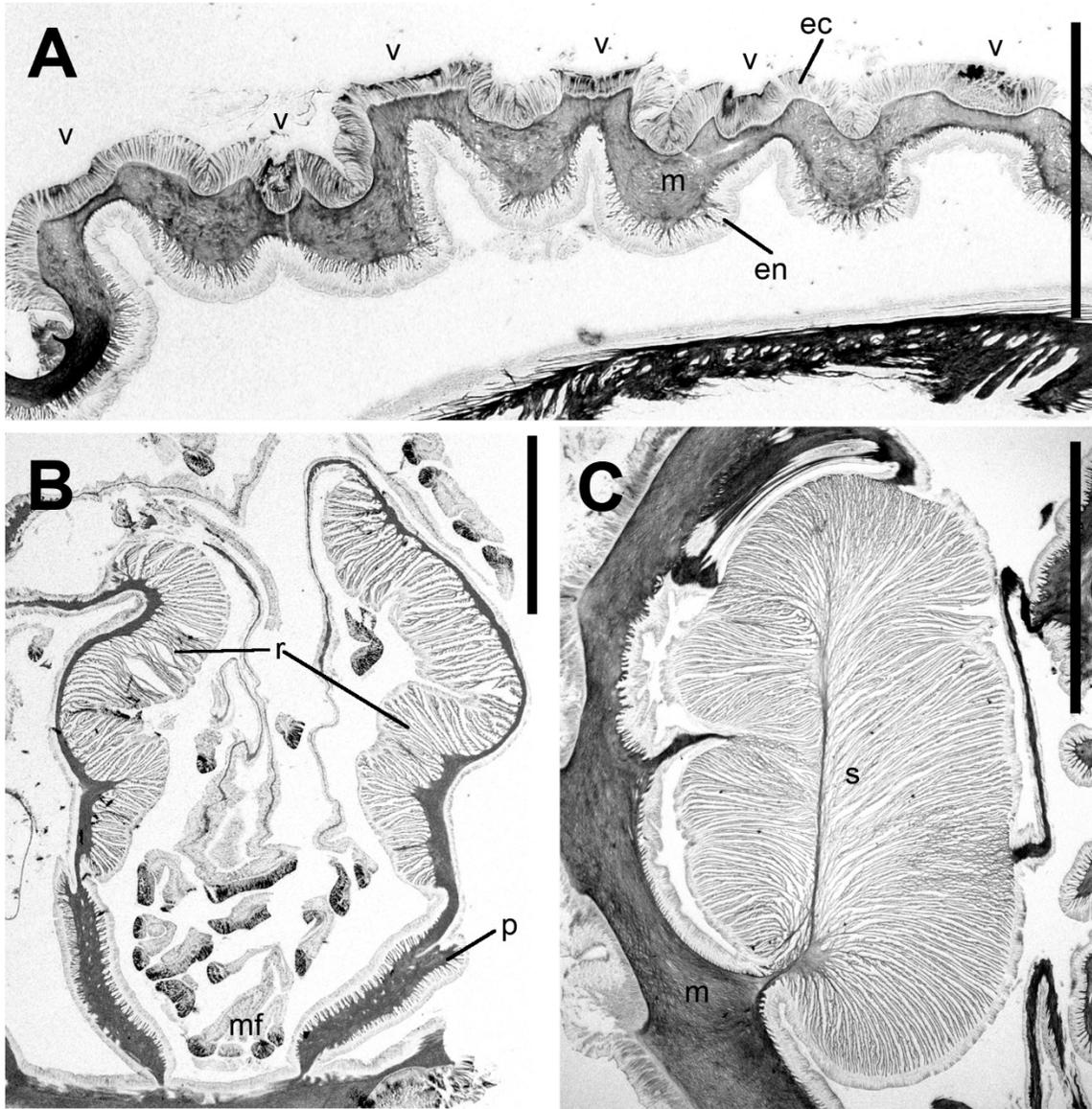


Figure 8 Histological sections of *Urticina fecunda*, comb. nov. A. Longitudinal section of body wall through a row of verrucae. B. Transverse section through mesenteries. C. Longitudinal section through marginal sphincter. ec—ectoderm, en—endoderm, m—mesoglea, mf—mesenterial filament, p—parietobasilar muscle, r—retractor muscle, s—marginal sphincter, v—verruca. Scale bars = 2 mm.

Tentacle length approximately 1/3 length of oral disk diameter, stout, with blunt tips.
Tentacles red, ringed with white basally, light rose at mid-point, tipped with white.
In preserved specimens, tentacles 1/2 length of oral disk radius, conical, longitudinally corrugated, same color as column. Radial muscles of oral disk and longitudinal muscles of tentacles ectodermal.

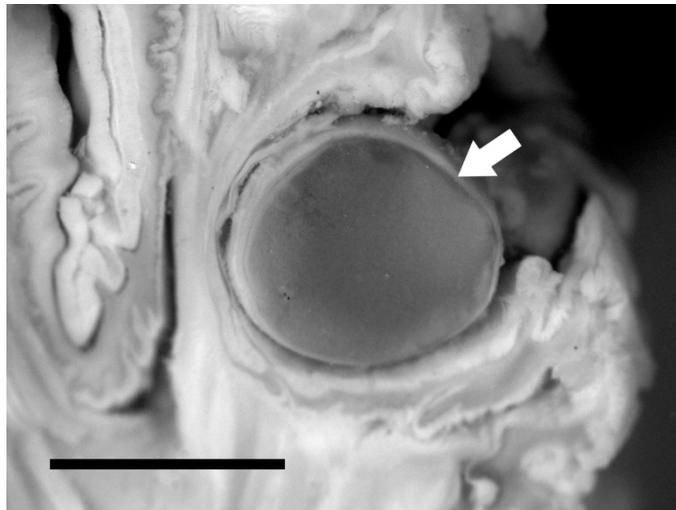


Figure 9 Longitudinal section through column of *Urticina fecunda*, comb. nov. (USNM 24885, syntype), showing the offspring (arrow) within a pit. Scale bar = 2 mm.

Internal anatomy: Actinopharynx bearing two siphonoglyphs without aboral prolongations. Marginal sphincter muscle endodermal, strong, circumscribed, oval, pinnate, symmetrical with respect to main lamella (Figure 8C). Parietobasilar muscles strong with short mesogleal free pennon, present from pedal disk to oral disk (Figure 8B).

Mesenteries in 3 cycles: first cycle decamerous, mostly perfect; subsequent cycles often irregular, with variable number of mesenteries. Marginal stomata present, no oral stomata seen. Directives and perfect mesenteries of 1st cycle sterile, other mesenteries may bear large yolky eggs or planulae, approximately 2 mm in diameter. All specimens examined either female or sterile. Retractor muscles strong; diffuse to restricted (Figure 8B). Same number of mesenteries distally and proximally.

Cnidom: Spirocysts, basitrichous isorhizas (basitrichs), micobasic *p*-mastigophores. (See Table 2 for size and distribution). Basitrichs of the column occur in two non-overlapping size ranges (Figure 10A): the larger (Figure 11G) occur throughout column whereas the smaller (Figure 11H) are associated only with verrucae.

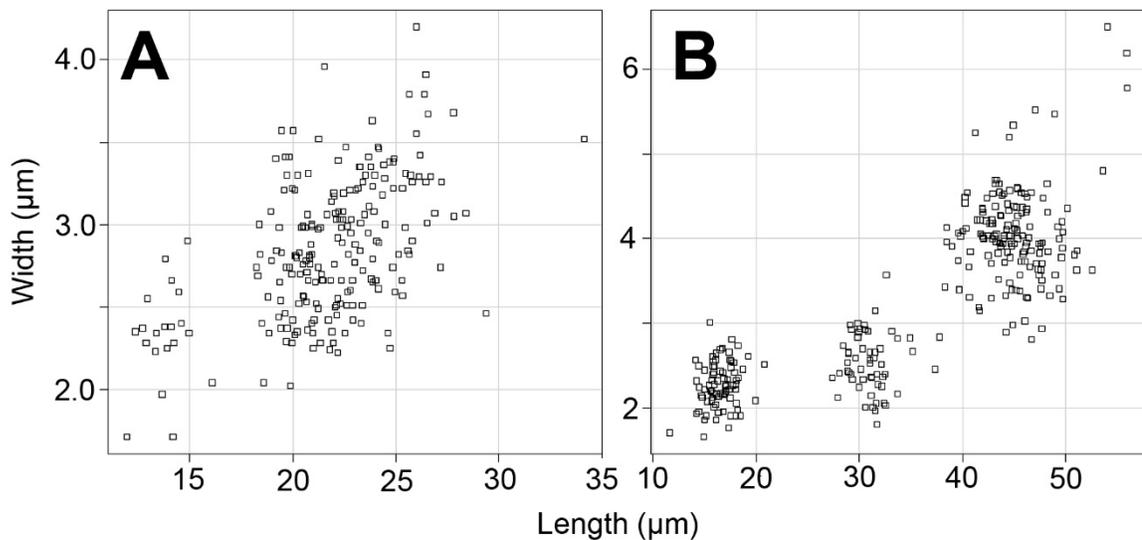


Figure 10 Scatter plot of basitrich length and width of *Urticina fecunda*, comb. nov. A. Column. B. Mesenterial filament.

The actinopharynx of recently collected specimens contain numerous microbasic *p*-mastigophores (Figure 11E), but the only evidence of these in the type material are opaque, globular masses of the approximately expected size and shape (likely discharged capsules).

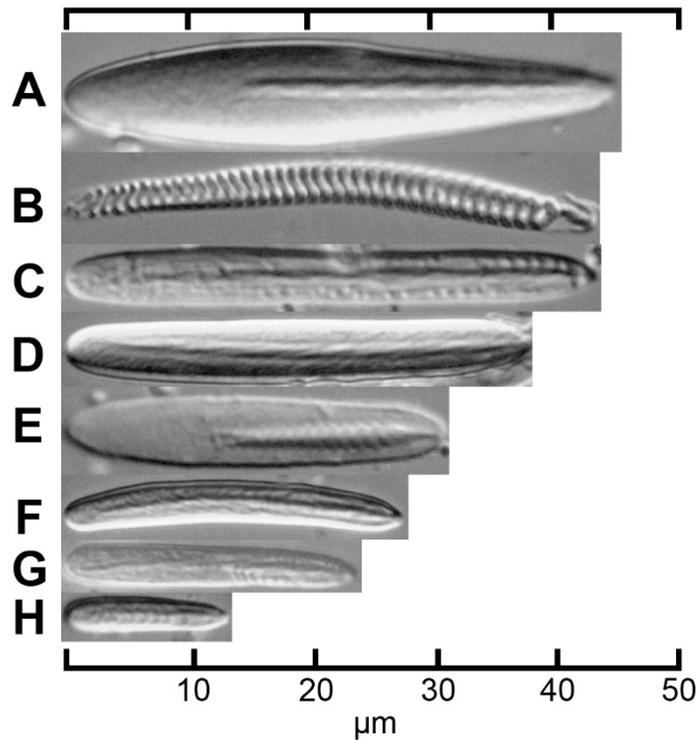


Figure 11 Cnidae of *Urticina fecunda*, comb. nov. A-microbasic *p*-mastigophore of mesenterial filament, large type, B-spirocyst, C-largest size basitrich of mesenterial filament, D-basitrich of actionpharynx, E-microbasic *p*-mastigophore of mesenterial filament, small type, F-basitrich of tentacle, G-basitrich of column, large type, H-basitrich of column, small type.

In mesenterial filaments of the type material, these cnidae are recognizable but frequently broken. Basitrichs occur in three size classes in the mesenterial filaments (Figure 10B).

Due to the presence of a large, condensed plug of mesenterial filament in the actinopharynx of preserved specimen YPN 34734 (*Epigonactis fecunda* syntype), a significant amount of contamination is suspected in the actinopharynx nematocyst measurements for that specimen (see Figure 12). This problem did not occur in both tissue types since mesenterial filament not in contact with actinopharynx tissue was available.

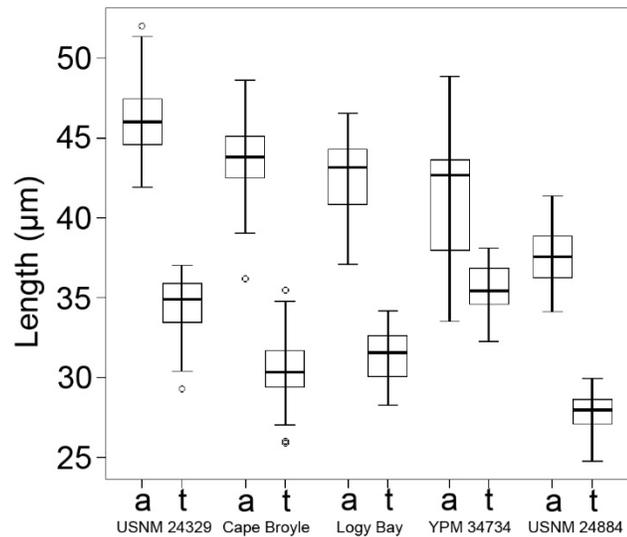


Figure 12 Boxplot diagram comparing basitrich lengths between actinopharynx (a) and tentacle (t) tissues among five specimens of *Urticina fecunda*, comb. nov., named by collection lot number or locality (if not deposited in a museum collection).

Habitat and distribution: Attached to hard substrata, generally with the pedal disk partly covered by sand or small pebbles. In some cases, only crown of tentacles visible over substrate. Specimens may be found at base of large rocks. The newly collected specimens were found between 10 and 20 m depth in habitats of low and medium energy. Sympatric species include *Aulactinia stella* (Verrill, 1864) and *Urticina felina* (Linnaeus, 1761).

Previous collections of *Urticina fecunda*, comb. nov., were made in deep water (below 275 m, to at least 366 m), whereas our new collections are from less than 20 m, indicating that this species has a wide bathymetric range (i.e. is not restricted to the bathyal zone). The present study also extends the geographic distribution further to the east, as we find *U. fecunda* along the Avalon Peninsula (southeastern Newfoundland).

Behavior and feeding: Generally the tentacles of *Urticina fecunda*, comb., nov. remain deployed in the water column 24 h a day and most days of the year. The only exception occurs after ingestion of a large prey item, when the sea anemone may remain retracted (at least partly) for some 24 – 36 h. Live specimens in captivity feed on a variety of prey, including small fish, shrimp, amphipods, sea urchins and also react positively to particulate organic matter.

Reproduction: This species initially broods zygotes to larvae inside its body cavity, and thereafter broods larvae externally to juveniles. In early June, females release internally brooded planulae that attach just underneath the crown of tentacles where they complete their development into juveniles. The larvae and early juveniles are maintained in the upper part of the column (about 3–6 rows of propagules) each in an individual pit (Figures 7, 9). Their attachment to the column is initially localized (Figure 7B) and they eventually spread out to form a collar (Figure 7D), either on their own or assisted by the female. No planula was ever found on the surrounding substrate after planulation events. Planulae become juveniles with fully developed tentacles about 4 months post release. At that stage, they are able to feed directly on suspended particles. Development of juveniles appears to be relatively synchronous, most of them growing visible tentacles inside a 1–2 week period. Juveniles detach from the females after about seven months (at a size of 2–3 mm pedal disk diameter), all inside a short period (between 1–5 d), and are easily found scattered on the rocks and pebbles surrounding the females for another eight months. Their growth seems slow; they reach about 4–5 mm (pedal disk diameter) at 12 months post release.

Discussion

The genus *Epigonactis* was erected by Verrill (1899) for two nominal species that he described from the northwestern North Atlantic: two externally brooding specimens from Nova Scotia assigned the binomen *Epigonactis fecunda*, and one non-brooding specimen from southwestern Newfoundland that he called *Epigonactis regularis*. As noted by Carlgren (1901), these two putative species are inseparable based on the anatomical

descriptions. Indeed, the primary feature distinguishing them appears to be the presence of brooded offspring in *E. fecunda* and lack thereof in *E. regularis*. Observations reported here show that the difference in reproductive condition (including presence or absence of brooding pits in the column of the adult) between *E. fecunda* and *E. regularis* could be explained by a different time of collection in relationship with the season of reproduction, but collection data for the syntypes do not include time of year. Our examination of the type material of *Epigonactis fecunda* (syntypes USNM 24329 and YPN 34734) and *Epigonactis regularis* (holotype USNM 24885) confirm their synonymy.

Carlgrén's (1901) suggestion that *Epigonactis* is congeneric with *Epiactis* was widely adopted by subsequent authors (e.g. Stephenson 1918, 1922; Dube 1974; Sebens 1998) although he later (1921) cautioned that the species should only be placed in the genus *Epiactis* "provisionally." Regardless, his 1949 survey listed the species as *Epiactis fecunda*. Carlgrén (1901) also considered *Leioterialia spetsbergensis* Kwietniewski, 1898 to be invalid, noting that: 1) among specimens in the type series were individuals of *Urticina crassicornis* (Müller, 1776) and 2) another specimen among the types was probably a specimen of *Epigonactis fecunda*: an assertion he justified through reference to a single externally brooded offspring (Carlgrén 1901, 1921) and the smooth column of that brooding individual (Carlgrén 1921). Carlgrén was mistaken in the last point: smoothness of the column is a feature that would differentiate *Epigonactis fecunda* from Kwietniewski's brooding specimen rather than unite them. Present examination of two syntype specimens of *L. spetsbergensis* (SMNH lot 5692) confirm the smooth nature of the column in specimens of the type series, although neither one can be confirmed as the

brooding individual. That individual apparently forms the basis for the inclusion of northern Europe in distribution data for “*Epiactis fecunda*” in databases such as European

Tissue	Cnida type	Min L	Max L	Mean L	SD L	Min W	Max W	Mean W	SD W	N	#
Column	Basitrich (large)	18.23	34.14	23.73	4.66	2.02	4.2	2.91	0.4	198	5/5
Column	Basitrich (small)	11.93	16.09	13.81	0.98	1.97	2.79	2.33	0.3	20	5/5
Tentacle	Basitrich	24.75	38.09	31.45	3.18	2.01	4.29	2.78	0.4	178	5/5
Tentacle	Spirocyst	21.1	61.46	38.08	8.92	1.66	3.08	2.33	0.3	108	5/5
Actinophx.	Basitrich	33.52	52.03	42.35	4	2.46	6.09	4.45	0.7	148	5/5
Actinophx.	Microbasic p-mastigophore (small)	28.22	35.9	31.61	1.8	5.01	7.88	6.08	0.7	55	2/5
Actinophx.	Microbasic p-mastigophore (large)	48.2	49.31	48.75	0.78	6.27	6.7	6.48	0.3	2	1/5
Actinophx.	Spirocyst	32.09	48.28	38.22	5.45	1.97	3.2	2.53	0.4	7	1/5
Mesenterial Filament	Basitrich (small)	11.61	20.8	16.53	1.47	1.66	3.01	2.27	0.3	78	5/5
Mesenterial Filament	Basitrich (medium)	27.42	35.18	30.87	1.67	1.81	3.57	2.53	0.3	55	5/5
Mesenterial filament	Basitrich (large)	37.38	55.95	44.96	3.36	2.46	6.5	4.03	0.6	157	5/5
Mesenterial Filament	Microbasic p-mastigophore (large)	40.94	56.57	48.04	3.15	3.33	10.82	7.48	1.4	41	5/5

Table 2 Cnida location, type, length (L, μm), width (W, μm), standard deviation (SD), number measured (N), and proportion of *Urticina fecunda*, comb. nov., in which cnida type was found (#).

Register of Marine Species (Costello *et al.* 2011), but considering Kwietniewski’s (1898) comment explicitly noting a lack of verrucae or “warts” (“Das Mauerblatt ist vollständig

warzenlos ...” p.135) and Carlgren’s (1901, 1921) similar comments, the specific identity of the brooding individual among the *L. spetsbergensis* series remains unknown, and the range of *Epigonactis fecunda* has not yet been confirmed outside of the northwestern North Atlantic Ocean.

Though verrucae on the column and the decamerous arrangement of tentacles and mesenteries preclude this species from being a member of *Epiactis* as that genus is currently understood (Verrill 1869a; Stephenson 1918, 1922; Carlgren 1921, 1949), those features are consistent with the generic diagnosis of *Urticina*, as are sterility of the directive mesenteries and the non-overlapping size distribution of basitrichs in the tentacles, where they are shorter, and actinopharynx, where they are longer (Carlgren 1921; Hand 1955) (Figure 12). The latter feature is commonly used to differentiate *Urticina* from *Cribrinopsis* Carlgren, 1921 a genus in which the basitrichs of the tentacles and actinopharynx have overlapping length distributions between these tissues (Carlgren 1921; Hand 1955; Sanamyan and Sanamyan 2006).

Urticina fecunda, comb. nov., represents the first known externally brooding species of *Urticina* and brings the current number of species assigned to the genus (Fautin 2011) to 12. There are currently no externally brooding species reported to be sympatric with *U. fecunda*, which means that this life history feature effectively distinguishes the latter from co-occurring species. Outside of its brooding season, however, other characters must be used to differentiate *U. fecunda* from other northwestern North Atlantic species of the genus. *U. fecunda* is easily differentiated from *U. crassicornis* by the strongly verrucose

column of the former compared with the weak or absent verrucae of the latter. In live animals, the color of *U. fecunda* (bright pink column scattered with white spots and silver lines at the base of the tentacles) and the adherence of gravel, sand, and debris at the base of the column differentiate this species from *U. felina*, where the column has a uniform colour, varying from beige-grey to dark red with longitudinal grooves extending from pedal disk to base of tentacles. The pedal disk in *U. fecunda* does not form any rolls and is round compared to *U. felina* where the pedal disk spreads out to become larger than the oral disk. Due to the loss of coloration, preserved non-brooding specimens of *U. fecunda* are most easily differentiated from those of sympatric *U. felina* by nematocysts.

Basitrichs in the tentacles of *U. felina* are 39.64–58.56 μm (N=80), longer than those of *U. fecunda* (see Table 2). Column tissue of *U. felina* lacks the small size class of basitrich found in *U. fecunda*, and *U. felina* has two comparatively small size classes of basitrich in the mesenterial filament (12.8–14.75 μm N=10 and 19.79–25.01 μm N=30) compared with three in *U. fecunda* (Table 2). Lastly, the actinopharynx of *U. felina* has only one size range of microbasic *p*-mastigophore (36.47–44.62 μm N=24) which nearly spans the gap between the two size ranges of this cnida type in *U. fecunda* (see Table 2). Presence of verrucae on the column separates *U. fecunda* from other externally brooding species that occur elsewhere in northern temperate and arctic seas such as species of *Epiactis* which exhibit hexamerous mesentery and tentacle arrangement and a smooth column. Our observations of living animals shed new light on hypotheses regarding how the offspring become attached to the adult anemone. Verrill (1899) characterized externally brooding species as “incubating their eggs externally.” Carlgren (1901) expressed strong

skepticism of the idea that pre-planular offspring could move from the gastrovascular cavity of an adult to its column, but two methods involving actions of the parent have since been described. In *Epiactis prolifera* Verrill, 1869a, a mass of mucus and eggs or zygotes are expelled from the parent's mouth and moved to, and over the oral margin by bulging of the pharynx and mouth. Some of them adhere to the column as they drift downwards (Dunn 1975b). Uchida and Iwata (1954) described a similar process in *Cnidopus* (= *Bunodes*) *japonicus* (Verrill, 1869b), but Nishihira and Ishimura (2003) documented the direct placement of offspring into an annular groove on the column of the adult in that species. In any case, the embryo never becomes a free-swimming, ciliated planula (Dunn 1975b; Uchida and Iwata 1954), and the capability for independent dispersal is limited by the locomotive mode of most sea anemones (crawling via the pedal disk). Here we give the first account consistent with Carlgren's (1901) hypothesis: offspring are first protected internally until the planula stage, and then exit the adult, moving to their external place of development on the column. Brooded, free-swimming offspring are known in other species, including *Actinia equina* (Linnaeus, 1758), *Urticina coriacea* (Cuvier, 1798), and *U. felina* (Chia and Rostron 1970; Hand 1955; Mercier *et al.* 2011), but in these species, the released larvae do not attach to the external column of an adult. Which ecological factors promote the retention and brooding of offspring that are ostensibly capable of planktonic dispersal is unknown.

Chapter 4: Revision of North Pacific *Epiactis* species.

Introduction

The intertidal zone of the North Pacific rim hosts multiple species of sea anemone (Cnidaria: Anthozoa: Actiniaria) that brood their offspring. In these species, the offspring are retained within or upon the body through development to a fully formed polyp. This definition excludes offspring that are released as larvae or embryos. The genus *Epiactis* Verrill, 1869a is notable among actinarians for its relatively high proportion of such species and for the diversity of strategies by which its members brood: members of *E. arctica* Verrill, 1868 and *E. marsupialis* Carlgren, 1921 brood offspring within individual pits that form by invagination of the column, whereas the offspring of members of *E. prolifera* Verrill, 1869a and *E. lisbethae* Fautin and Chia, 1986 are fully exposed upon the surface of the parent. In other species, such as *E. fernaldi* Fautin and Chia, 1986, offspring are retained within the gastrovascular cavity of the adult and released as juveniles via the mouth of the parent. The genus is widely distributed globally, but most species-rich in the Pacific (7 of 18 species). In the North Pacific, external brooding is reported in *E. prolifera* (see Verrill 1869a), *E. lisbethae* (see Fautin and Chia, 1986), and *E. japonica* (see Uchida 1934); internal brooding has been reported in *E. fernaldi* (see Fautin and Chia 1986) and *E. ritteri* (see Hand and Dunn 1974). The specific identity of brooding individuals in the last species is contested (Sanamyan and Sanamyan 1998),

reflecting different interpretations of features not described in the original description, such as adherence of debris to the column.

Epiactis ritteri Torrey, 1902 was described from specimens collected from Popof Island, Alaska. These individuals were not brooding externally at the time of collection. The description did not include any mention of internal brooding or provide any details about brooding behavior or structures, except in noting the absence of external brooding as a point of contrast with the externally brooding species *E. prolifera*. Reproduction is a seasonal phenomenon in many species (Shick 1991), however, and presence or absence of offspring may be circumstantial. None of the subsequent publications mentioning *E. ritteri* from Alaska (Carlgren 1934, 1947, 1950) discussed individuals that brooded offspring internally or externally.

Dunn (1972) identified internally brooding anemones from Bodega Bay, California as belonging to *Epiactis ritteri*. This was the first report of the species from outside Alaska and the first account of brooding for *E. ritteri*. The redescription of *E. ritteri* by Hand and Dunn (1974) based on internally-brooding specimens from California and Oregon is consistent with previous work (Torrey 1902; Carlgren 1934) with respect to features like marginal sphincter morphology, mesenterial arrangement, and concentration of holotrichous isorhizae (holotrichs) in the basal column. Hand and Dunn (1974) also provided important details not mentioned in earlier work, most notably the adherence of foreign material to the column and the batteries of holotrichs in the distal column.

Sanamyan and Sanamyan (1998) contested the identification by Dunn (1972) and by Hand and Dunn (1974), arguing that the details noted by Hand and Dunn (1974) but not

described by previous workers were indicative of this misidentification, and suggesting that the southern specimens studied by Hand and Dunn (1974) actually represent a new species.

The identity of *Epiactis ritteri* was also challenged on the basis of its similarity with *E. japonica*, an externally brooding species from Japan. Uchida (1934, 1938), Uchida and Iwata (1954), and Carlgren (1940, 1947, 1950, 1952) hypothesized that the Alaskan individuals of *E. ritteri* belonged to *E. japonica*. Sanamyan and Sanamyan (1998) concurred, considering *E. japonica* and *E. ritteri* synonymous based on externally brooding specimens from the Commander Islands, Kamchatka Peninsula, Kurile Islands, and Sea of Japan. Further complicating this situation, Uchida (1934, 1938) and Uchida and Iwata (1954) used the name *E. prolifera*, which belongs to an Eastern Pacific species, to refer to what was subsequently identified as *E. japonica*, raising the possibility that *E. ritteri* is a junior synonym of *E. prolifera*. Synonymy between *E. prolifera* and *E. ritteri* was invariably rejected by Carlgren (e.g., 1940, 1947, 1950) and eventually dropped by Uchida (see Dunn 1972).

The taxonomic history of *Epiactis ritteri* is further complicated by Carlgren's (1934) establishment of the genus *Cnidopus* Carlgren for that species. The genus *Epiactis* is characterized by a smooth column (Verrill 1869a, Stephenson 1922, Carlgren 1921, 1949, Hand 1955) that may be modified temporarily: grooves or pits may form during external brooding in some species, but these are not present in animals without attached offspring. In the Alaskan specimens of *E. ritteri* that Carlgren (1934) examined, there is a band of holotrich-packed protuberances surrounding the base of the animal. This band of

holotrich-dense protuberances, present even when an animal is not externally brooding, is the diagnostic feature of *Cnidopus* (see Carlgren 1934). Holotrichs in the column is one of the features that differs between the specimens of *E. ritteri* from Alaska and those from California and Oregon: Carlgren (1934, 1945) described the column of the Alaskan specimens as being free of holotrichs except in these protuberances, whereas Hand and Dunn (1974) reported batteries of holotrichs throughout the column of animals from California and Oregon.

Different authors have attributed more or less significance to the occurrence of holotrichs and to the basal protuberances in the column, and have thus considered *Cnidopus* a valid genus or a junior synonym of *Epiactis*. Fautin and Chia (1986) considered these two features to be independent, citing the presence of holotrichs elsewhere on the column of their *E. ritteri* specimens and in species lacking basal protuberances such as *E. prolifera*. Fautin and Chia (1986) asserted that neither was of generic significance and synonymized *Epiactis* and *Cnidopus*. Sanamyan and Sanamyan (1998) concurred with Carlgren (1934, 1945) in considering *Cnidopus* a valid genus. Like Carlgren (1934, 1945), Sanamyan and Sanamyan (1998) found holotrichs associated only with the basal protuberances and therefore considered the protuberances as a single complex structure analogous to the acrorhagi that characterize e.g., *Anthopleura* Duchassaing de Fonbressin and Michelotti, 1860, or *Actinia* (Linnaeus, 1767), and thus sufficient for generic distinction (Sanamyan and Sanamyan 1998).

The taxonomic debate about the importance of these features has resulted in both “*Cnidopus*” and “*Epiactis*” being used in reference to the North American species:

Zamponi and Excoffon (1988, 1995) use *C. ritteri* whereas Edmands (1995, 1996) uses *E. ritteri*; Song (1992) and Daly *et al.* (2002) use *E. japonica* whereas Ishimura and Nishihira (2002), Kostina *et al.* (2006), Yanagi and Daly (2004), and Uchida and Soyama (2001) use *C. japonicus*. England (1992) suggested establishing a new genus for the Japanese species to account for a modification of the column commonly found in it, namely the “spherules” occupying the middle region of the column (Figure 13). Fautin *et al.* (2007) listed the genus *Cnidopus* as “valid,” but at the time of writing, it is considered “not valid” in Fautin’s (2013) online database. Here we follow the most recent genus-level revision (Fautin and Chia, 1986) and use *Epiactis* for *E. ritteri* and *E. japonica*, but acknowledge that the synonymy of these genera requires evaluation.

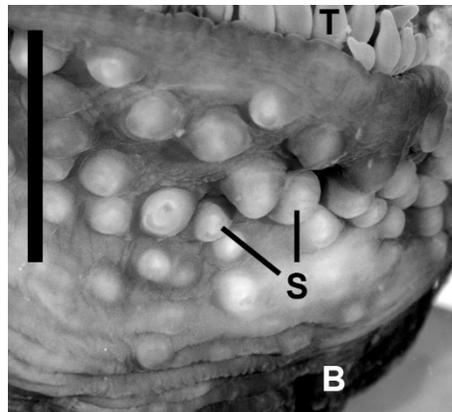


Figure 13 Preserved *Epiactis japonica*. B, band of basal vesicles; S-mid-column spherules, T-tentacles.

The confusion and debate about the attributes of *Epiactis ritteri* cannot easily be resolved because the critical species lack designated type specimens and vouchers. Neither Torrey (1902), nor Carlgren (1934), nor Hand and Dunn (1974) identified individual specimens upon which their respective findings were based. Therefore, it is impossible to consult the original material to adjudicate conflicting taxonomic hypotheses. We attempt to mitigate this obstacle in two ways: by making new collections from type localities and other locations across the range in which these species ostensibly occur, and by borrowing specimens which have been determined to species by the authors of these studies. Furthermore, we timed sampling in Alaska to coincide with the brooding season of the Japanese species to investigate Uchida's (1934) concern that Torrey's (1902) omission of brooding in *E. ritteri* was simply due to seasonality of reproduction. As a result, we are able to identify characteristics by which the species can be individuated. We redescribe the internally brooding species *E. ritteri sensu* Hand and Dunn (1974) as *E. handi* sp. nov. We redescribe *E. ritteri* to eliminate confusion about the attributes of that species, document its mode of brooding, provide additional localities, and discuss attributes that may serve to maintain species boundaries between *E. ritteri* and *E. japonica*.

Materials and Methods

Individuals of species of *Epiactis* were observed in the field and collected from their substrate at low tide with tools such as forceps and a spatula. Collection sites span the North Pacific (Figure 14). Specimens were preserved in 10% buffered formalin sea water solution (4% formaldehyde) or 95% ethanol. Newly collected material has been deposited

search pattern described by Williams (1996). Cnida terminology follows Mariscal (1974). Measurement range, mean, and standard deviation are reported and intended for qualitative comparison among species rather than for statistical evaluation (see Williams 1996, 1998, 2000).

Results

Systematics:

Order ACTINIARIA Hertwig, 1882

Suborder Enthemonae Rodríguez *et al.* 2014

Superfamily Actinioidea Rodríguez *et al.* 2014

Family ACTINIIDAE Rafinesque, 1815

Genus *Epiactis* Verrill, 1869a

Epiactis handi sp. nov.

(Figures 15–18)

Synonymy:

Cnidopus ritteri (Dunn, **1972**: 139, 147–153, 156, 166, 173. –Hand and Dunn, **1974**:

187–192. –Fautin and Chia, **1986**: 1665, 1673. –Zamponi and Excoffon, **1988**: 43, 45–48, **1995**: 5. –Zamponi **1989**: 1–43, **1993**: 13, 15, **2000**: 48, 49.)

Epiactis ritteri (Fautin and Chia, **1986**: 1665, 1670, 1673. –Fautin, *et al.*, **1987**: 76–77. –

Zamponi and Excoffon, **1988**: 48. –Edmands and Fautin, **1991**: 59. –Edmands,

1995: 723–731, **1996**: 228–235, –Edmands and Potts, **1997**: 485–489, 491–495.)

Pro parte *Epiactis ritteri* (Kostina, **1988** 16, 18.)

Non *Cnidopus ritteri* (Carlgren, **1934**: 350, 351, **1945**: 10, **1949**: 62. –Uchida and Iwata, **1954**: 224.)

Non *Epiactis ritteri* (Torrey, **1902**: 393–394. –Stephenson, **1922**: 274. –Carlgren, **1934**: 351, **1947**: 92, **1950**: 138, **1952**: 387. –Uchida, **1934** 18, 23, 29, 30. –Fautin, Zelenchuk, *et al.*, **2007**: 198.)

Non *Epiactis riterii* [sic] (Uchida and Iwata, **1954**: 224.)

Type specimen

One specimen in formalin. North of Copper Bay, Moresby Island, British Columbia, Canada; 53.2°, -131.8°; intertidal; [AMNH5293]; coll. P. Larson / M. Daly, 17 October 2013.

Other material

Three specimens in formalin and 10 microscope slides. West side of Yakan Point, Graham Island, British Columbia, Canada; intertidal; [California Academy of Science (CAS) 61560]; coll. D.G. Fautin / R.W. Buddemeier, 19 August 1986.

Five specimens in formalin and 13 microscope slides. Tow Hill, Graham Island, British Columbia, Canada; intertidal; [CAS 61565]; coll. D.G. Fautin/R.W. Buddemeier, 19 August 1986.

Four specimens in formalin. Tow Hill, Graham Island, British Columbia, Canada; 54.1°, -131.8°; intertidal; [AMNH5291]; coll. P. Larson / M. Daly, 20 October 2013.

Two specimens in formalin. Skidegate, Graham Island, British Columbia, Canada; 53.3°, -132.0°; intertidal; [AMNH5292]; coll. P. Larson / M. Daly, 24 October 2013.

Five specimens in formalin and 9 microscope slides. Gray Bay, East coast of Moresby Island, British Columbia, Canada; intertidal; [CAS 61562]; coll. D.G. Fautin / R.W. Buddemeier, 21 August 1986.

One specimen in formalin. North of Copper Bay, Moresby Island, British Columbia, Canada; 53.2°, -131.8°; intertidal; coll. P. Larson / M. Daly, 17 October 2013.

Three specimens in formalin and 10 microscope slides. Newcombe Harbour entrance, McCutcheon Point, Pitt Island, British Columbia, Canada; 53.7°, -130.1°; intertidal; [CAS 61564]; coll. D.G. Fautin, 12 August 1986.

Three specimens in formalin and 10 microscope slides. North Rocks of West Beach, Calvert Island, British Columbia, Canada; 51.65°, -128.15°; intertidal; [CAS 61567]; coll. D.G. Fautin, 4 August 1986.

Seven specimens in formalin and 13 microscope slides. Kooryet Bay, Principe Channel, Banks Island, British Columbia, Canada, 53.33°, -129.87°; intertidal; [CAS 61561]; coll. D.G. Fautin, 11 August 1986.

Thirteen specimens in formalin and 10 histological microscope slides. Wadham's Landing, British Columbia, Canada; 51.48°, -127.53°; intertidal; [CAS 61580]; coll. D. Fautin, 2 August 1986.

Nineteen specimens in formalin and 26 microscope slides. Off Astoria, Oregon, USA; depth: 73 m; [Smithsonian Institution National Museum of Natural History (USNM) 51607]; coll. L. Marriage, 6 December 1947.

One specimen in formalin. NW of Bodega Marine Laboratory, Sonoma Co., California, USA; intertidal; [CAS 103187]; coll. S. Edmands. No date given.

Four specimens in formalin. West of Bodega Marine Laboratory, Bodega Head, Sonoma County, California, USA; intertidal; [CAS 10747]; coll. D.F. Dunn, 1 December 1979.

Diagnosis:

Gonochoric *Epiactis*; females brood internally. Column adherent with sand, bits of shell, or other debris. Ectoderm of column with specialized regions of densely packed, nonglandular cells. Limbus longitudinally furrowed, with abundant holotrichous isorhizae.

Description:

Color: Column variable: maroon, salmon, brown, olive green, teal. Limbus usually with lighter longitudinal stripes (solid or interrupted), sometimes with bluish ring overlaying background color (Figure 15A). Column often finely flecked with spots slightly darker than background color. Oral disk may differ in color from column and tentacles, frequently purple; monochrome (Figure 15B), radially striped, or with chevrons at base of tentacles (Figure 15C). Tentacles colored as column, unornamented except by chevrons at base in some specimens.

Column: Widest at base, to 80mm in diameter, smooth distally, longitudinally wrinkled proximally. In preserved specimens, annular wrinkles near limbus may intersect with longitudinal wrinkles, producing irregular protuberances of various size and shape:

squarish bulges, oblong ridges, or slight mounds. These bulges may be less pronounced, variable in shape, or absent across the circumference of a single specimen (Figure 16B).

Distal column smooth, lacks verrucae or vesicles, but ectoderm and ectodermal surface of mesoglea finely corrugate in histological section (Figure 17A). Holotrichs concentrated in clusters on and between basal protuberances, may be present in batteries distally on column (See Hand and Dunn, 1974). Regions of tightly packed, nonglandular cells (Figure 17C) which lack cnidae and resemble suckers sensu Stephenson (1928) present throughout ectoderm of column, possibly responsible for adherence of foreign materials (Figure 15A).

Oral disk and tentacles: Mouth slightly raised. Oral disk bare medially; tentacles restricted to periphery. Radial muscles of oral disk ectodermal, circular muscles endodermal. Tentacles hexamerously arranged, slender, conical, terminally perforate, slightly blunt at tips (Figure 15B, C). Circular muscles of tentacle endodermal, longitudinal muscles ectodermal.

Internal anatomy: Two siphonoglyphs extend aborally beyond actinopharynx. Up to 5 cycles of mesenteries: first and second cycles perfect but sterile, third through fifth imperfect but fertile. Gonochoric. Oral and marginal stomata.

Retractor muscle restricted to diffuse. Parietobasilar muscle strong, with thin flap extending freely from mesentery (Figure 17A). Marginal sphincter endodermal, strong, pinnate, lateral lobe extends proximally.

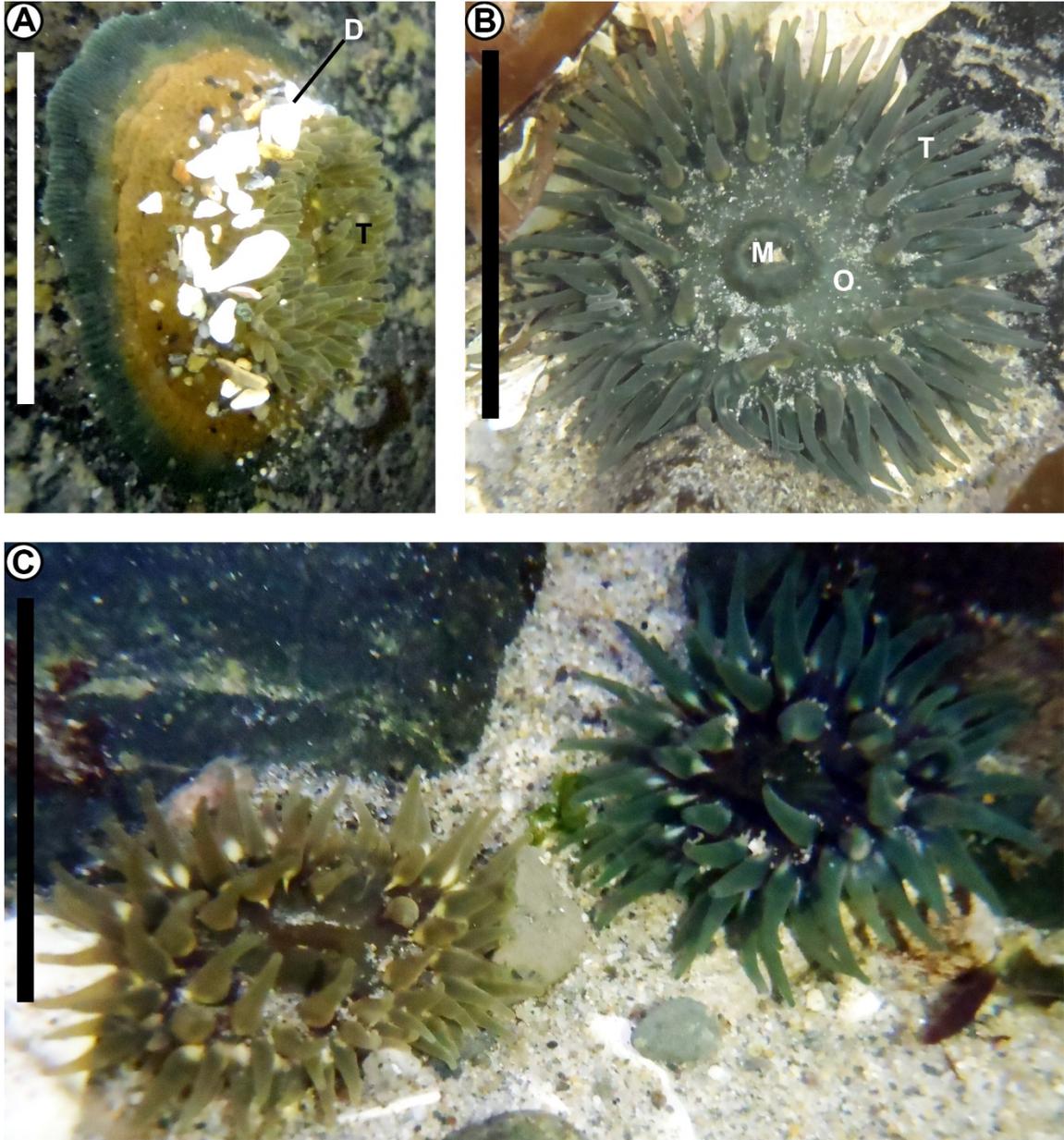


Figure 15 *Epiactis handi* in situ: (A) partially contracted specimen on rock with adherent debris and bluish basal ring; (B) oral disc of expanded individual; (C) variously coloured individuals attached to rocks slightly below sand surface. D, adherent debris; M-mouth, O-oral disk, T-tentacle.

Cnidom: Basitrichs, holotrichs, microbasic *p*-mastigophores, spirocysts (Figure 6). See Table 3 for size and distribution.

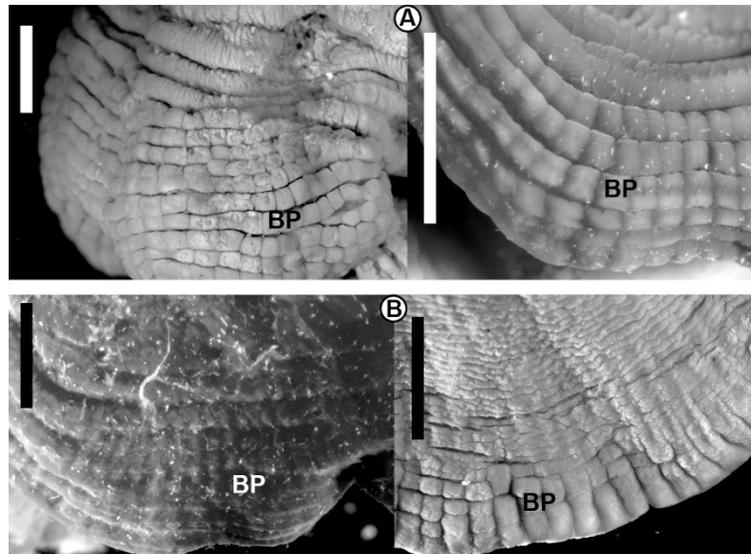


Figure 16 Features of the proximal column in *E. ritteri* and *E. handi*. (A) two specimens of *E. ritteri* showing the continuous band of regular, squarish protuberances characteristic of the species; (B) two specimens of *E. handi* showing discontinuous, irregular sculpturing at the base of the column. BP, basal protuberances.

Life history: Internally broods relatively few offspring, but to large size (e.g., one preserved adult in USNM lot 51607 with 37mm pedal disk diameter contained one offspring; it had 10mm diameter pedal disk diameter and at least 44 tentacles). Asexual reproduction via budding reported in two individuals from British Columbia, Canada

(Zamponi and Excoffon 1988); none of our specimens have scars or other indications of this process.

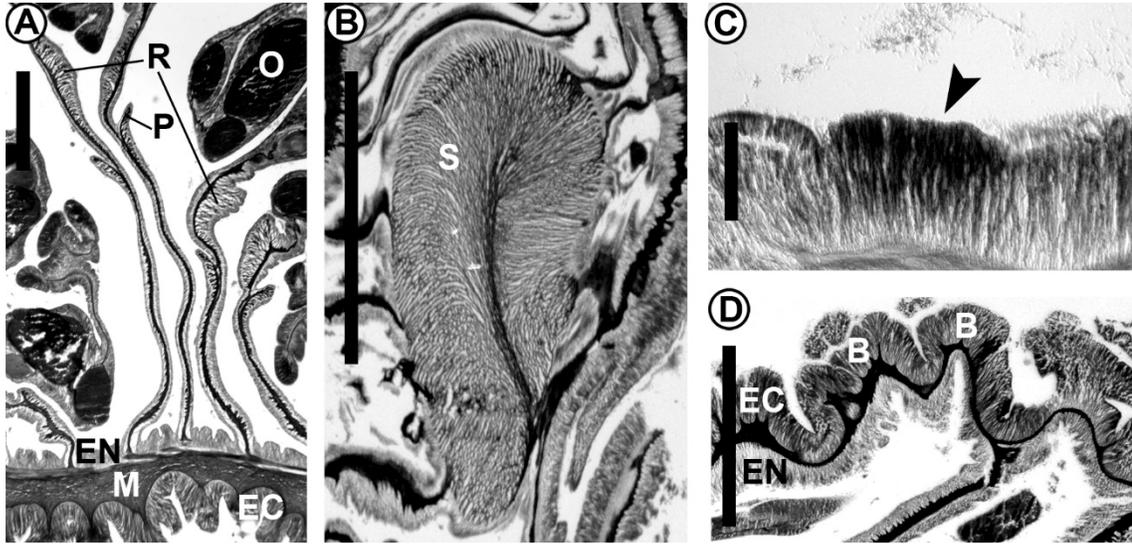


Figure 17 Histological sections of *E. handi*: (A) cross-section through column and mesenteries; (B) longitudinal section through sphincter; (C) specialized region of ectoderm (arrow); (D) irregular protuberances near limbus. B, basal protuberance; EC, ectoderm; EN-endoderm, M-mesoglea, O-oocyte, P-parietobasilar muscle, R-retractor muscle, S-sphincter. Scale bars: A, 0.5 mm; B, 2 mm; C, 100 μ m; D, 1 mm.

Habitat, geographical, and bathymetric distribution: Eastern Pacific Ocean: Central California to Graham Island, Canada. Occurs from mid intertidal to at least 73m; attaches to various substrates (e.g., cobbles and boulders in sandy intertidal; mollusc shells, wood, skate egg cases in deeper waters). May be fully exposed upon bare rock at low tide or partially covered with base attached to rocks beneath sand surface. Often most abundant actiniarian in cobble habitats in Haida Gwaii (Queen Charlotte Islands), British Columbia, where sympatric species typically include *Urticina coriacea* (Cuvier, 1798),

U. clandestina Sanamyan *et al.*, 2013 and occasionally include *Anthopleura artemisia* (Pickering in Dana, 1846) and *A. elegantissima* (Brandt, 1835). Less abundant and more cryptic towards southern end of range (see Hand and Dunn, 1974).

Remarks: Hand and Dunn (1974) reported holotrich batteries distally in the column in addition to those on and among basal protuberances. Hand and Dunn (1974) suggested the possibility of sequential hermaphroditism based on a single relatively large male specimen, but present material includes large males and females.

Etymology: *Epiactis handi* honors Dr. Cadet Hand, former director of the Bodega Marine Laboratory, and senior author of the first report of this species (as *E. ritteri*, see Hand and Dunn 1974).

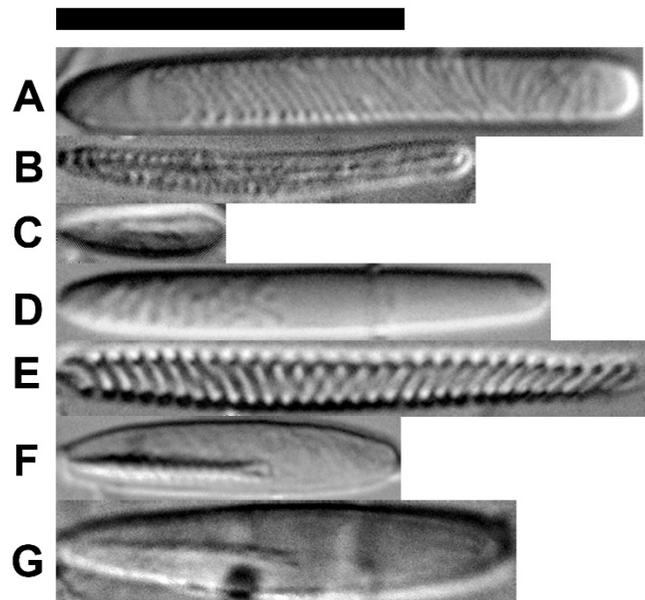


Figure 18 Cnidae of *E. handi*, *E. ritteri*, and *E. japonica*. Letters refer to types in Table 3. Scale bar: 20 μ m.

Epiactis ritteri Torrey, 1902 (Figures 16, 19–21)

Synonymy

Cnidopus ritteri (Carlgren, **1934**: 350, 351, **1945**: 10, **1949**: 62. –Uchida and Iwata, **1954**: 224.)

Epiactis ritteri (Torrey, **1902**: 393–394. –Stephenson, **1922**: 274. –Carlgren **1934**: 351, **1947**: 92, **1950**: 138, **1952**: 387. –Uchida, **1934**: 18, 23, 29, 30. –Kostina, **1988**: 16, 18. –Fautin, Zelenchuk, *et al.*, **2007**: 198.)

Epiactis riterii [sic] (Uchida and Iwata, **1954**: 224.)

Pro parte *Epiactis ritteri* (Kostina, **1988**: 16, 18.)

Non *Cnidopus ritteri* (Dunn, **1972**: 139, 147–153, 156, 166, 173. –Hand and Dunn, **1974**: 187–192. –Fautin and Chia, **1986**: 1665, 1673. –Zamponi and Excoffon, **1988**: 43, 45–48, **1995**: 5. –Zamponi, **1989**: 1–43, **1993**: 13, 15, **2000**: 48, 49.)

Non *Epiactis ritteri* (Fautin and Chia, **1986**: 1665, 1670, 1673. –Fautin *et al.*, **1987**: 76–77. –Zamponi and Excoffon, **1988**: 48. –Edmands and Fautin, **1991**: 59. –Edmands, **1995**: 723–731, **1996**: 228–235, –Edmands and Potts, **1997**: 485–489, 491–495.)

Material examined

One specimen in 75% ethanol and 13 microscope slides. Sumisher[sic] Island, Kuril Islands, Sea of Okhotsk, Russia); [CAS 31235]; coll. unknown, on or prior to 14 May 1982.

Two specimens in formalin and 35 microscope slides. Palisade Rocks, Adak Island, Alaska, USA; 51.9°, -176.6°; intertidal; [AMNH5294]; coll. P. Larson, 13 April 2012.

Three specimens in formalin and 54 microscope slides. Breakwater, Adak Island, Alaska, USA; coordinates: 51.9°, -176.6°; intertidal; [AMNH5295]; coll. P. Larson, 14 April 2012.

Two specimens in 75% ethanol. Dutch Harbor, Unalaska Island, Alaska, USA; [CAS 3437]; coll. unknown, 26 May (Year not given).

Two specimens in 70% ethanol and 15 microscope slides (St. George Island, Alaska, USA; intertidal); [USNM 53298]; coll. G D. Hanna, 13 June 1914.

Three specimens in 70% ethanol St. George Island, Alaska, USA; [USNM 43065]; coll. G D. Hanna, 20 August 1914.

Five specimens (plus brooded offspring) in 70% ethanol and 15 microscope slides (St. Paul Island, Alaska, USA); [USNM 32969]; coll. W. L. Hahn, 7 March 1911.

Four specimens in formalin and 41 microscope slides; Mill Bay, Kodiak Island, Alaska, USA; 57.8°, -152.3° intertidal; [AMNH5296]; coll. P. Larson, 19 April 2012.

Three specimens in formalin and 40 microscope slides; Mill Bay, Kodiak Island, Alaska, USA; 57.8°, -152.3° intertidal; [AMNH5297]; coll. P. Larson, 19 April 2012.

Two specimens in 70% ethanol and 15 histological microscope slides. St. George Island, Alaska, USA; intertidal; [USNM 53298]; coll. G D. Hanna, 13 June 1914.

Three specimens in 70% ethanol. St. George Island, Alaska, USA; [USNM 43065]; coll. G D. Hanna, 20 August 1914.

Five specimens (plus brooded offspring) in 70% ethanol and 15 histological microscope slides. (St. Paul Island, Alaska, USA); [USNM 32969]; coll. W.L. Hahn, 7 March 1911.

Three specimens in formalin and 4 histological microscope slides. Torch Bay, Gulf of Alaska, Alaska, USA; [CAS 52757]; coll. M. Dethier, 1978.

One specimen in formalin and 4 histological microscope slides. Torch Bay, Gulf of Alaska, Alaska, USA; [CAS 52756]; coll. M. Dethier, 1978.

Diagnosis

Gonochoric *Epiactis*; females brood offspring externally. Column cylindrical and smooth except for broadly flared, flattened limbus ornamented with bands of square-based protuberances densely packed with holotrichous isorhizae.

Description

Color

Bright orange, maroon, brown, or slightly reddish-purple (Figure 19). Adjacent individuals commonly differ in color. Monochromatic, excepting one individual in Kodiak with white chevrons at base of tentacles. Inflated tentacles may appear lighter than column, due to slight translucence of thinned tissue.

Column

Moderately sized, 18–53mm basal diameter preserved, wider in life. Limbus much wider than rest of column, flared out and flattened significantly in live animal (Figure 19).

Smooth in histological section, except for a proximal band of regularly arrayed non-adhesive square vesicles, (Figure 19B, C, Figure 16A) involving all three layers of body

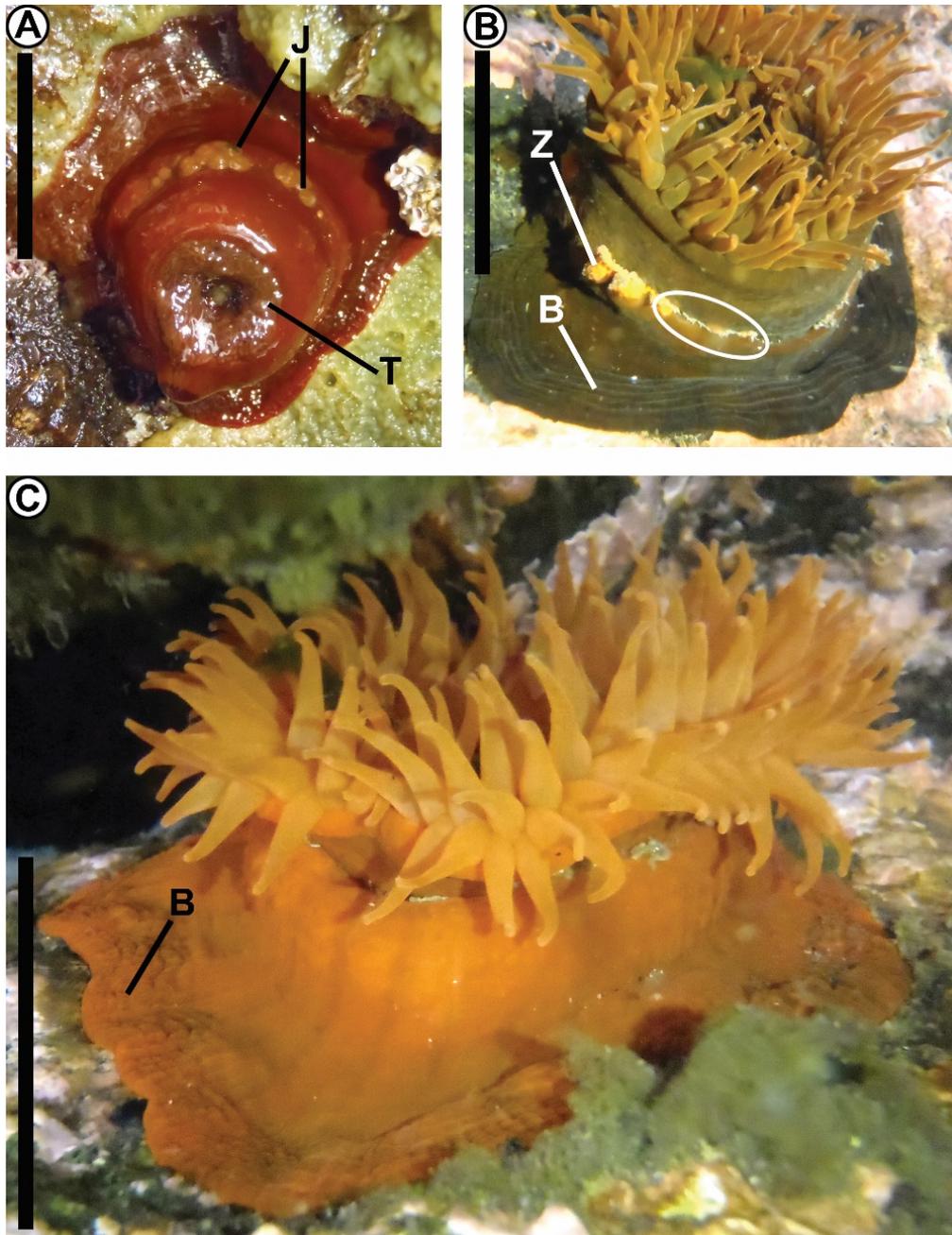


Figure 19 *Epiactis Ritteri* in situ: Three colour morphs in various habits and brooding state: (A) fully emerged, partially contracted individual at low tide with several fully exposed offspring, maroon type; (B) submerged individual bearing zygotes or embryos in a groove which has begun closing (circled area), brown type; (C) submerged individual without offspring, orange type. B-basal band of vesicles, J-juvenile, T-tentacles, Z-zygotes or embryos. Scale bars: 2 cm.

wall (Figure 20C) and armed with abundant holotrichs (Figure 20D, Figure 18A).

Mesoglea thicker than ectoderm and endoderm, except at basal vesicles. Oral disk and tentacles

Oral disk bare medially; tentacles restricted to peripheral half. Radial muscles of oral disk ectodermal. Tentacles hexamerously arranged, conical, terminally perforated, slightly blunt at tips. Circular muscles of tentacle endodermal, longitudinal muscles ectodermal.

Internal anatomy

Usually two siphonoglyphs, some specimens reported with one (Torrey 1902). Up to 5 cycles of mesenteries, first and second perfect but sterile, third through fifth imperfect but fertile. Gonochoric. Oral and marginal stomata present.

Retractor muscle thin, diffuse. Parietobasilar muscle strong, stout, with broad free flap extending freely from mesentery (Figure 20A). Marginal sphincter endodermal, strong, pinnate, with lateral lobe extending proximally, lamellae slightly anastomosing. (Figure 20B).

Cnidom

Basitrichs, holotrichs, microbasic *p*-mastigophores, spirocysts (Figure 18). See Table 3 for size and distribution.

Life History

Females brood offspring externally in early spring (observed in March, April). Offspring initially within sealed annular groove formed by invagination of the column, later groove disappears and offspring are exposed on surface of parent. Offspring brooded until they have at least 3 cycles of mesenteries; brooded offspring capable of feeding (copepod observed in gastrovascular cavity of brooded offspring). Mesenteries of brooding females fertile.

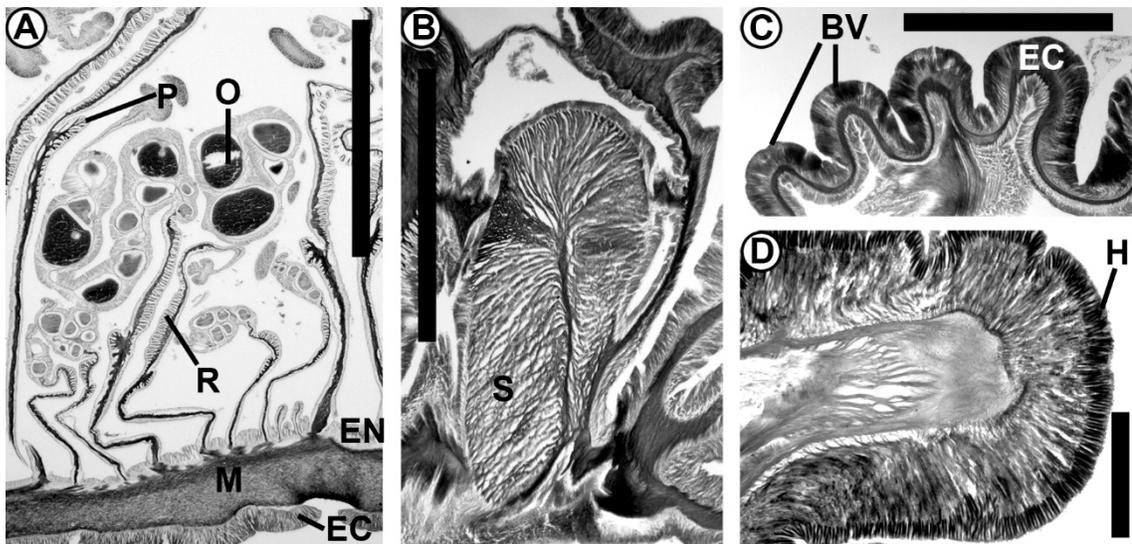


Figure 20 Histological sections of *E. Ritteri*: (A) cross section through column of female; (B) longitudinal section through sphincter; (C) longitudinal section through basal vesicles; (D) basal vesicle detail with stained holotrichs. BV-basal vesicle, EC-ectoderm, H-holotrichs, M-mesoglea, O-oocyte, P-parietobasilar muscle, R-retractor muscle, S-sphincter. Scale bars: 2 mm.

Habitat and range

Moderately abundant in mid to low rocky intertidal but patchily distributed. Often on bare rock or sponge in tidepools or attached to large (immobile) submerged rocks, but tolerant of full emersion (individuals observed on vertical rock outcrop approximately 60cm above water surface). Sympatric with *Urticina grebelnyi* Sanamyan and Sanamyan, 2006, *Metridium* sp., and *Anthopleura artemesia*. Aleutian Islands, Bering Sea, Gulf of Alaska, USA.

Remarks

Torrey (1902) did not identify type specimens for *Epiactis ritteri*, and we found no material of an age to have been studied as part of the original description. However, H.B. Torrey was listed as the determiner of USNM 52043, collected in March, 1911.

One specimen of *Epiactis ritteri* [AMNH 5295] deviates from the description above in two respects. First, the sphincter is attached to the mesoglea of the body wall along its entire length, rather than only at the base of a main lamella (as shown in Figure 20 B). A main lamella is lacking in this instance, and the branches of the muscle appear to originate from the mesoglea of the body wall along the length of the sphincter. Second, on the column, distal to the brood groove, there are close-set, longitudinal columns of low, small mounds. The endoderm of this region is smooth, but the ectoderm has wrinkles that correspond to the mounds. In some places, the mounds co-occur with a narrow invagination of the endoderm into the mesoglea; this invagination usually does not extend beyond the ectodermal surface of the adjacent column tissue. These are most prominent in the middle, diminishing towards the parapet and brood groove. These

bumps differ from the spherules described below in *E. japonica* (Figure 13) in that they contain mesoglea, are highly ordered in longitudinal rows, densely spaced, and irregular and in section. In *E. japonica*, there are large, circular, widely spaced spherules, variable in number from zero to several dozens, usually scattered irregularly on all parts of the column except the basal protuberances. High, broad evaginations of all three layers of the body wall result in a distinct, hollow, bulbous protuberance in *E. japonica*.

DISCUSSION

Distinguishing E. handi from E. ritteri.

Our findings confirm the assertion of Sanamyan and Sanamyan (1998) that Hand and Dunn's (1974) "redescription" of *Epiactis ritteri* was actually an account of a new species, which we describe and name here as *Epiactis handi* sp. nov. This may also be the *Epiactis* species from Oregon which Carlgren (1952, p. 387) mentioned (but did not describe or name) as having "2 embryos provided with several tentacles in the gastral cavity."

Epiactis handi and *E. ritteri* have much in common with respect to size, distribution of cnidae, and internal anatomy, but differ in several ways. The most readily apparent difference in living specimens are in the column: a specimen of *E. ritteri* lacks adherent foreign material and bears offspring, whereas one of *E. handi* has adherent material and lacks externally brooded offspring. However, as these are seasonal or situational differences (e.g., adherent material is easily lost in preserved specimens, brooding is seasonal and restricted to females in *E. ritteri*) we discuss more consistently evident

anatomical differences below. The anatomy of the base of the column has contributed to the confusion about the identity of *Epiactis ritteri* and to disagreement about the necessity of a separate genus for species having bumpy (versus smooth) columns. Torrey (1902) noted “protuberances” caused by transverse and longitudinal wrinkles in the column, but Carlgren (1934) described them more fully, determining that they harbor a type of nematocyst (originally called an “atrich,” now considered a holotrich: see Westfall, 1965) restricted to the limbus in *E. ritteri*. In *E. ritteri*, these protuberances are non-adhesive and satisfy Carlgren’s (1949) definition of “vesicle” but not “verruca.” Hand and Dunn (1974: p. 190) use the term “verruca” for the basal protuberances of what we describe as *E. handi*, citing their adhesive nature and Carlgren’s (1952: p. 387) use of that term for them. In that instance, however, Carlgren was actually referring to the relatively large, hemispherical mid- column spherules found in the Japanese species *E. japonica* (Figure 13), and he goes on to note their *absence* in *E. ritteri*: “The protuberances which usually occur in the middle part of the body are, as far as I can see, verrucae though they probably are not very adhesive. ... Neither Torrey nor I myself have found any verrucae in the middle region of the body...” We do not consider the basal protuberances in *E. ritteri* to be verrucae, preferring the terms vesicle or basal protuberance. We concur with Hand and Dunn (1974) that the column of *E. handi* is adhesive (see below), but we fail to find hollow outgrowths with the histological characteristics ascribed to verrucae (Stephenson 1928; Häussermann 2004: see also Figure 17). The basal column of *E. handi* is smooth or with longitudinal wrinkles

Table 3. Cnida types found in *E. Ritteri* and *E. handi*. Bas-basitrich, Spir-spirocyst, Holo-holotrich, M. p-m-microbasic p-mastigophore, L-length, W-width, \bar{x} -mean, N-total number of capsules measured, S-proportion of examined specimens with cnida type, SD-standard deviation; Letters after nematocyst type correspond with types depicted in Figure 18.

Table 3

Tissue & Type	<i>Epiactis Ritteri</i>				<i>Epiactis handi</i>			
	(Range of L) X (Range of W)	(\bar{x} L \pm SD L) X (\bar{x} W \pm SD W)	n	S	(Range of L) X (Range of W)	(\bar{x} L \pm SD L) X (\bar{x} W \pm SD W)	n	S
COLUMN								
Bas. C	(9.34 – 11.30) X (1.68 – 2.40)	(10.3 \pm 0.54) X (1.96 \pm 0.2)	12	6/17	----	----	-	-
Bas. B	(14.07 – 35.36) X (1.47 – 4.60)	(20.75 \pm 2.08) X (2.47 \pm 0.37)	1099	17/17	(12.07 – 33.01) X (0.9 – 4.32)	(18.88 \pm 2.39) X (2.2 \pm 0.36)	368	5/5
Holo. A	28.11 – 47.32 X (3.01 – 5.60)	(37.32 \pm 3.32) X (4.32 \pm 0.49)	458	17/17	(17.73 – 41.43) X (2.69 – 5.12)	(32.58 \pm 6.14) X (3.9 \pm 0.47)	139	5/5
TENTACLE								
Bas. C	(9.20 – 13.48) X (1.55 – 2.40)	(11.64 \pm 1.15) X (1.98 \pm 0.25)	15	6/17	----	----	-	-
Bas. B	(16.35 – 35.03) X (1.22 – 3.90)	(27.72 \pm 2.88) X (2.48 \pm 0.39)	634	17/17	(19.91 – 29.89) X (1.51 – 3.24)	(24.67 \pm 2.34) X (2.3 \pm 0.32)	157	5/5
Spir. E	12.78 – 49.13 X (1.37 – 4.00)	(30.47 \pm 7.23) X (2.65 \pm 0.49)	450	17/17	(14.58 – 33.24) X (1.47 – 3.01)	(24.23 \pm 3.98) X (2.2 \pm 0.33)	110	5/5
Holo. D	(12.78 – 38.91) X (2.28 – 5.70)	(29.27 \pm 3.32) X (3.55 \pm 0.53)	178	14/17	(20.12 – 31.18) X (2.12 – 3.86)	(25.72 \pm 2.49) X (2.9 \pm 0.36)	101	4/5
CATCH TENTACLE								
Bas. B	(29.42 – 31.39) X (2.05 – 2.80)	(30.14 \pm 1.08) X (2.51 \pm 0.4)	3	1/1	----	----	-	-
Spir. E	39.48 X 2.62		1	1/1	----	----	-	-
Holo. A	(24.36 – 33.37) X (2.2 – 3.40)	(29.51 \pm 2.46) X (2.7 \pm 0.32)	40	1/1	----	----	-	-
ACTINOPHARYNX								
Bas. B	(12.33 – 35.60) X (1.55 – 4.70)	(31 \pm 2.94) X (2.91 \pm 0.46)	533	15/15	(21.4 – 31.64) X (2.12 – 4.09)	(26.93 \pm 2.18) X (3.1 \pm 0.37)	161	5/5
M. <i>p-m</i> . F	(17.82 – 27.40) X (2.66 – 6.00)	(21.93 \pm 2.11) X (4.33 \pm 0.59)	44	7/15	(18.98 – 24.11) X (3.03 – 4.46)	(21.49 \pm 1.73) X (4 \pm 0.36)	14	2/5
MESENTERIAL FILAMENT								
Bas. B	(12.72 – 29.68) X (1.29 – 3.90)	(19.43 \pm 3.03) X (2.39 \pm 0.4)	209	14/16	(10.25 – 28.29) X (1.32 – 4.5)	(20.98 \pm 3.25) X (2.4 \pm 0.41)	120	5/5
M. <i>p-m</i> . F	(14.8 – 28.68) X (2.54 – 6.20)	(21.03 \pm 2.85) X (4.24 \pm 0.52)	347	15/16	(15.39 – 27.12) X (2.8 – 5.26)	(20.72 \pm 2.14) X (4.2 \pm 0.5)	129	5/5
M. <i>p-m</i> . G	(12.24 – 35.00) X (2.00 – 5.80)	(24.23 \pm 2.73) X (4.01 \pm 0.53)	351	14/16	(19.45 – 33.06) X (2.83 – 5.59)	(24.76 \pm 3.91) X (3.9 \pm 0.66)	86	3/5

that sometimes intersect with annular wrinkles to form restricted protuberances.

Distributed among the protuberances and the rest of the column are sucker-like (*sensu* Stephenson 1928) regions of ectodermal tissue and batteries of holotrichs, however the latter are primarily found in the basal region.

The basal protuberances differ in arrangement and morphology between species. In *Epiactis handi*, they are irregular in size and shape, do not form a consistent band around the perimeter near the limbus, and primarily appear after death and fixation, if they are present at all (Figure 16B); slight basal longitudinal wrinkles may be evident in life. In contrast, in *E. Ritteri*, multiple, regular rows of fairly uniform, squared protuberances form a complete band around the base of the animal in life (Figure 19) and in preserved specimens (Figure 16A). The number of rows in a band of protuberances in *E. Ritteri* appears to be loosely correlated with size (0 rows in brooded offspring, 5–8 rows in smaller specimens from Adak and Kodiak, up to 16 rows in large specimens from Unalaska Island and the Pribilof Islands).

Two other differences in the column are evident in the histology of the ectoderm and mesoglea. First, in preserved specimens of *Epiactis handi*, the ectoderm and the ectodermal surface of the mesoglea are finely wrinkled and branched (Figure 17A), whereas in *E. Ritteri* these layers of the body wall are smooth (Figure 20A) even in contracted animals. Second, the column ectoderm of *E. handi* has regions of densely packed cells which lack nematocysts (Figure 17C), but these are not found in *E. Ritteri*. Hand and Dunn (1974) proposed that these specialized areas are comparable to “suckers” *sensu* Stephenson (1928) and may facilitate sand grain adhesion to the column.

Live animals of the two species differ in color and are easily distinguished. *Epiactis ritteri* is monochromatic: tentacles, oral disc, and column are uniform orange, maroon, brown, or slightly reddish-purple (Figure 19). The single deviation from this pattern was one specimen with white chevrons at the base of the tentacles. In contrast, in *E. handi*, the basal column and oral disk is more variable, including multiple colors in a single individual, sometimes similar in color and pattern to *E. prolifera* with radiating white lines (Dunn 1972, Hand and Dunn 1974), and sometimes with a blue ring at the limbus.

A fuller account of *E. ritteri*.

Our new material allows us to flesh out and complete earlier accounts of *Epiactis ritteri*. Most critically, we lay to rest controversy about the mode of brooding: *E. ritteri* broods externally (Figure 19A), with some specimens retaining young within a tightly sealed brood-groove (Figure 21). Brood grooves have been reported in other externally brooding species (e.g., *Cricophorus nutrix*: Carlgren, 1924; *Epiactis mortensenii*: Carlgren, 1924; *Epiactis japonica*: Ishimura and Nishihira, 2002) and are formed by a depression that encircles the column of the adult. The brood groove has been hypothesized to provide protection or aid in the retention of brooded offspring, especially in adults which are not attached to horizontal surfaces (Ishimura and Nishihira 2002). In Uchida and Iwata's (1954) study of development in *E. japonica*, they did not discuss brood grooves or their formation but noted that embryos were "nearly wrapped with the mother's ectoderm" (Uchida and Iwata 1954, p. 222). Ishimura and Nishihira (2002) described the process of groove formation in *E. japonica*, noting that groove formation occurs simultaneously with offspring deposition: the mouth is extended toward the column causing a fold in the

column to form, and this fold is retained as the mouth revolves around the animal and deposits offspring into it. The offspring may be completely covered by the lower edge of this groove, but can also be visible when the tentacles of the parent are retracted (Ishimura and Nishihira 2002). *Aulactinia sulcata* (Clubb, 1902) exhibits enigmatic structures in the column that are superficially similar to the brood groove described here for *E. Ritteri*. Clubb (1902) described an annular groove that formed from a series of pits in the column (apparently prior to the appearance of offspring); these pits expand and merge through breakdown of the walls separating them, resulting in four to six large chambers invaginating proximally from a shallower annular groove. His longitudinal sections of a chamber appear similar to Figure 21 except notably, the meeting ectodermal layers at the edge of the groove are rounded. Carlgren and Stephenson (1929) disputed Clubb's (1902) description, instead describing a single invagination which soon bifurcates and extends its branches in opposite directions to form a single, nearly toroidal brood chamber within the adult. Dunn (1983) mentioned a specimen of *A. sulcata* with a single invaginated brood chamber that was empty and had been perforated by the mesenteries. In any case, the basic nature of the brood groove in *E. Ritteri* is different from the structures found in *A. sulcata* in that it is a single, continuous depression in the column rather than a series of distinct, invaginated pouches.

Although spawning has not yet been observed in *Epiactis Ritteri*, late-stage embryos can be found within a brood groove that is similar to that described for *E. Japonica*. In *E. Ritteri*, the groove is tightly sealed by the time offspring within the groove form clumps of small orange spheres 0.5mm in diameter (Fig 7B), and the two ectodermal edges of the

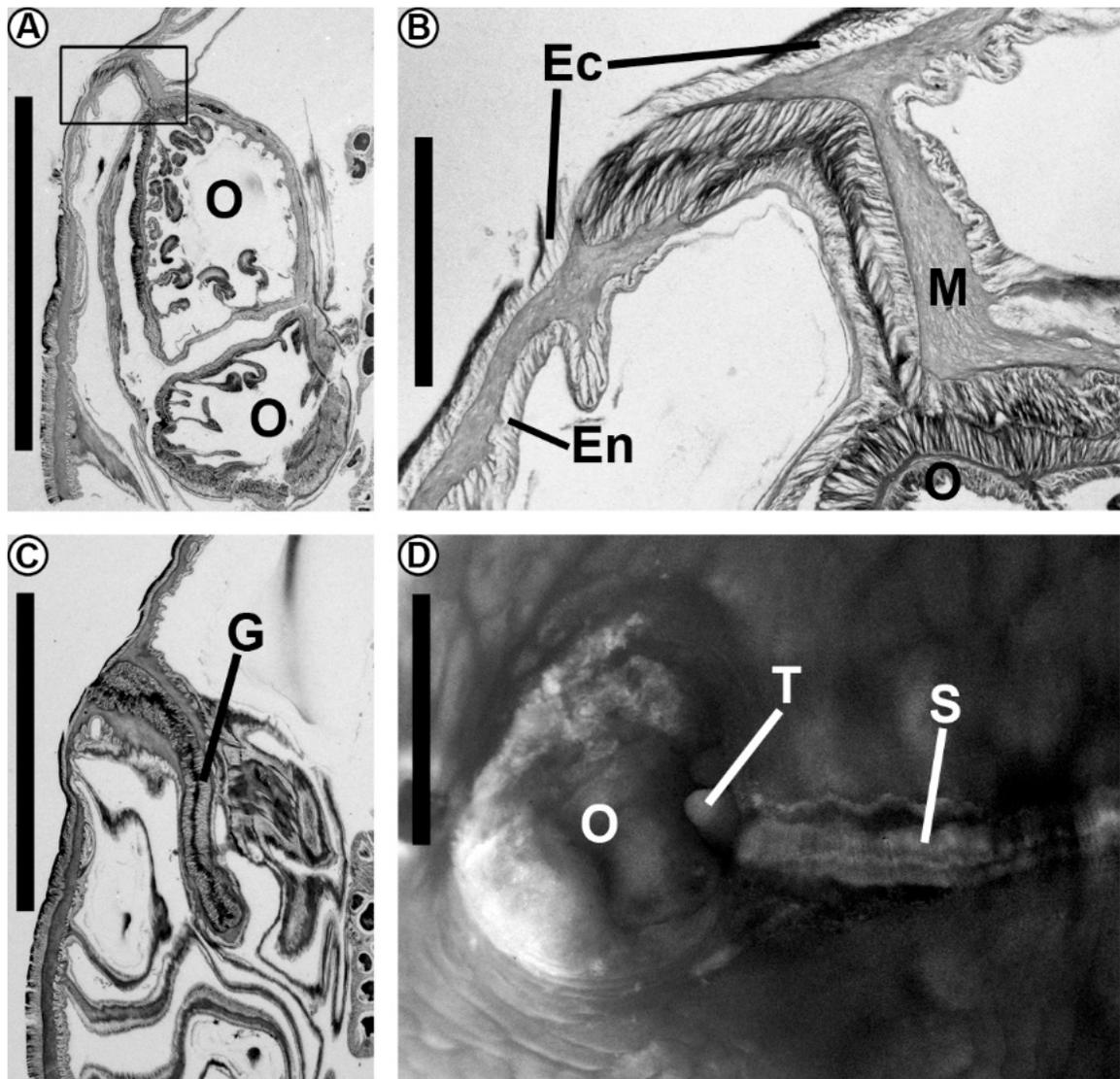


Figure 21 *Epiactis ritteri* Torrey, 1902: (A) longitudinal section through column of adult showing cross-section of brood groove containing two offspring; (B) detail of brood groove seal boxed area in (A); (C) collapsed groove in space between offspring; (D) external view of brood groove where seam has torn open to reveal an offspring. Ec-ectoderm, En-endoderm, M-mesoglea, G-groove, O-offspring, S-brood groove seam, T-tentacle (of offspring). Scale bars: A, C, D, 4 mm; B, 0.5 mm.

groove touch (Figure 21) . In this state, the column of the brooding adult has a smooth appearance in spite of being considerably folded. The opening to the groove is cryptic, evident only as a thin suture around the animal (Figure 21D). Because the groove is cryptic, several specimens which were initially thought to be non-brooding were revealed to bear offspring once dissected.

In histological section, the mesogleal and ectodermal layers near the opening of the brood groove appear to have been under tensile stress at fixation (Figure 21B), suggesting some mechanism by which the ectodermal surfaces adhere to one another. Gently probing the suture with a needle failed to separate ectodermal surfaces from one another in a preserved individual. In the spaces between adjacent offspring, the groove is completely collapsed upon itself (Figure 21C). In some specimens, the groove has been everted, probably during especially strong contractions of the adult during fixation. The everted groove is a somewhat globular ring of thin column tissue with offspring attached to bulges. In *Epiactis ritteri*, offspring remain fully enclosed within the groove even after they have developed tentacles and a pedal disk; most offspring were contracted and hard to evaluate, but an expanded individual in a forcibly everted groove had 18 tentacles). As the groove opens and begins to disappear, offspring remain on the adult, fully exposed to the environment, similar to the situation in *E. prolifera* (see Verrill 1869a). We have found a copepod in the gastrovascular cavity of an offspring on the (exposed) column of the parent, indicating exposed young are capable of feeding. Nonetheless, in the several examined specimens, histological sections reveal yolk granules in the gastrovascular cavity of exposed, attached offspring.

Individuals in which juveniles were fully exposed upon the adult had fewer (8–23) young than those which still held offspring within a groove (≥ 23 – ≥ 53). Counts of those in the latter state are limited to portions of the animal which have been dissected, or in which the groove has been everted, and thus are minimum numbers for those specimens. The difference may be due to juveniles having left the parent for independent life or having been washed off the adult once exposed. Closed brood grooves may contain hundreds of pre-juvenile offspring (prior to tentacle and pedal disk formation: i.e. zygotes or embryos).

In *Epiactis ritteri*, the offspring upon a brooding parent were all in a similar stage of development, but the broods of different adults from the same locality and collection date were at various stages, from zygote (or egg?) to juvenile with at least 3 cycles of mesenteries. Only (but not all) females brooded offspring and only a single male was collected in 2012. Borrowed material also contained only male or female individuals, thus there is yet no evidence for hermaphroditism in *E. ritteri*. Reproduction appears to occur on a seasonal basis, with all of an individual's efforts occurring in one event; this is in contrast with *E. prolifera*, in which individuals may reproduce continually throughout the year (Dunn 1975b). Despite the seasonality of reproduction, as the diversity of developmental stages seen within the same population indicates, reproduction is not highly synchronized between all individuals in a population. All adults collected by PL in Alaska were fertile in April regardless of reproductive state. Such overlapping of generations (i.e. brooded juveniles concurrent with developing oocytes in the parent) and

relative asynchrony within populations has also been described by Rodríguez *et al.* (2012b) in the externally brooding Antarctic species *E. georgiana* Carlgren, 1927. The duration and timing of brooding season for *Epiactis Ritteri* is unknown. In April 2012, the largest brooded offspring from Kodiak and Adak islands had a pedal disk diameter of 3.5mm (measured after fixation); the smallest were small spheres (eggs or early embryos) no larger than the oocytes still held within the parent (~0.5mm). A specimen from Alaska [USNM 52043] collected in March, 1911, bore (externally) much larger offspring, ranging in pedal disk diameter from 4.9 –16.5mm (preserved). This specimen was determined by Torrey (see above), but its reproductive state has not been acknowledged in any published account of *E. Ritteri*. This size discrepancy among broods also mirrors the findings of Rodríguez *et al.* (2012b) in *E. georgiana*.

In addition to providing details of reproductive biology, we provide the first thorough account of the cnidom of *Epiactis Ritteri*. Carlgren (1934) reported measurements of cnidae only for the column, tentacles, and actinopharynx, and did not specify the number of individuals studied or the number of capsules measured. Our measurements (Table 3) agree with his in general, except our size ranges are slightly broader and we include several rare or localized types he did not report. The column and tentacles of *E. Ritteri* both contain holotrichs, but these differ in morphology as undischarged capsules. Holotrichs of the tentacle (Figure 18D) have a thicker tubule visible only in the apex-end of the capsule when undischarged; the tubule may be coiled but is most often packed irregularly. In contrast, the holotrichs in the column (Figure 18A) have a thinner tubule which is visible throughout the capsule and is usually regularly coiled. The former appear

to correspond with the holotrichs figured in Fautin and Chia (1986), although Fautin and Chia (1986) made no distinction between those of the column and tentacle, and only depict one type. As Fautin and Chia (1986) reported for *E. lisbethae*, *E. fernaldi*, and *E. prolifera*, holotrichs in the tentacles of *E. ritteri* and *E. handi* are most abundant in the tentacles nearest the margin and may be rare or absent in a tentacle nearer the mouth. In a tentacle of one specimen of *E. ritteri*, we encountered very many holotrichs of the type normally found in the column (Figure 18A). Spirocysts were very rare in the sample from this tentacle. This is possibly similar to the abnormally long “catch tentacles” that Sanamyan and Sanamyan (1998, 2008, 2010) described occurring among the usual tentacle type in some of their specimens of *E. japonica*. In our material, however, there was no macroscopic indication (e.g., length, thickness) that the tentacle was different from others, and it was found only by chance. Re-inspection of all specimens of *E. ritteri* and haphazard resampling of tentacles yielded no more of this type of tentacle. We did, however, find several such tentacles in a preserved specimen of *E. japonica*, but in that case the “catch tentacle” type was distinct in being slightly longer, conical, and more opaque (tissue denser or thicker) in contrast to the tentacles with the more typical complement of cnidae, which were extremely contracted and more translucent. Differences in size and distribution of cnida among North Pacific *Epiactis* species are either very subtle (if not an artifact of sampling) or occur in cnida types that are highly variable in their distribution within a species. Thus, attributes of the cnidom are not practically useful for distinguishing these species. Whether certain types of cnidae that are not found consistently (e.g., microbasic *p*-mastigophore of the actinopharynx) are

missed due to true absence from particular specimens or patchy distribution within an individual is uncertain. Holotrichs can be found in the basal column and tentacles of *E. ritteri*, *E. handi*, *E. japonica*, *E. prolifera*, *E. lisbethae* (personal observations), however their relative abundance differs dramatically, being very common in the former three species, and less common in the latter two.

Distinguishing *E. ritteri* from *E. japonica*

Although *Epiactis japonica* and *E. ritteri* are similar in many respects, we note differences that argue for their continued recognition as separate species. Individuals of *E. japonica* are possibly protogynous (Uchida and Iwata 1954), but eventually become simultaneously hermaphroditic (Uchida and Iwata 1954, Ishimura and Nishihira 2002, Sanamyan and Sanamyan 2010), although gametes of a single type are spatially separated (on different mesenteries, or proximo-distally within a single mesentery) within the animal (Uchida and Iwata 1954). Torrey and Carlgren did not discuss sex allocation in discussion of mesenteric fertility in *E. ritteri*, but all material examined here exhibits the gonochoric condition. Because we find no hermaphrodites, even among the largest specimens, we think that simultaneous hermaphroditism in *E. ritteri* is rare or absent. The most apparent external differences between *Epiactis ritteri* and *E. japonica* are coloration and ornamentation of the column. Color variation is very low among the specimens we collected in Alaska: only 2–3 color varieties are present (bright orange, dark maroon, to dark purplish-brown). In the field, all individuals we saw were monochromatic, except one individual with white chevrons at base of its tentacles. In *E. japonica*, in contrast, color and patterning are extremely variable. In Hokkaido, we

observed individuals that vary from monochrome, to simple bicolored (tentacles differ from column), to irregularly streaked and mottled (column three or more colors). The oral disk of *E. japonica* is often radially striped with two or more colors. The background body colors of *E. japonica* commonly include red, maroon, pink, brown, tan, beige, green, and rarely bright blue. The monochromatic and relatively reduced suite of colors in *E. Ritteri* is consistent across the range from Kodiak to Adak, approximately 1700 km, following the Alaska Peninsula and Aleutian chain (see Lindeberg 2008 for images from intermediate sites; species identified as *E. prolifera*).

In our material of *E. japonica* (from Hokkaido, Japan), we found non-adhesive, hollow (i.e. involving all three layers of body wall), approximately hemispherical protuberances, on the middle and distal portion of the column of many of the smaller and all of the larger individuals (Fig 1). These were referred to as “papillae” by Uchida (1938), “verrucae” by Carlgren (1952), and “spherules” by England (1992) and Sanamyan and Sanamyan (1998). The one-third (22 of 71) of the collected individuals we examined that lacked such protuberances had a pedal disk diameter of 17mm or smaller (preserved); some individuals <17mm pedal disk diameter and all individuals larger than this size (up to ~54mm pedal disk diameter) possessed at least one (but usually many) such protuberances. Sanamyan and Sanamyan (1998) report individuals that they identify as *E. japonica* with and without mid-column protuberances co-occurring in the Commander Islands, but make no mention of relative sizes. We infer that this feature is acquired or becomes apparent as the animal grows. Noting the similarity in appearance between contracted, brooded offspring and mid-columnal spherules, England (1992) proposed that

these structures served as protective camouflage for brooded young. These spherules are absent in *E. Ritteri*: we do not see them in preserved specimens or live animals of any size, a conclusion that accords with Carlgren's (1952) note that neither he nor Torrey had observed them after examining "many" specimens.

The brood groove is different in *Epiactis japonica* and *E. Ritteri*. Although life history, reproduction, and development has been thoroughly studied in *E. japonica* (e.g., Uchida and Iwata 1954, Ishimura and Nishihira 2002) no one has described such a sealed groove in this species. While offspring may be covered, initially, the groove that is formed in *E. japonica* remains a depression partially open to the environment and offspring are often visible as tiny spheres on the column (e.g., Uchida and Iwata 1954, Ishimura and Nishihira 2002). We find that in Alaskan *E. Ritteri*, the brood groove is sealed early, before larvae develop tentacles, and remains sealed even when the offspring have multiple cycles of tentacles.

Based on our observations, the Alaskan specimens of *Epiactis Ritteri* are distinct from the Japanese specimens of *E. japonica* in terms of sex allocation, characteristics of brooding, and some morphological features, but the variability present in *E. japonica* across its range complicates species identification based on external characters alone, especially if these species occur in sympatry, as Sanamyan and Sanamyan (1998) suggested happens in the Kurile Islands. Sanamyan and Sanamyan (1998) were initially inclined to consider individuals with and without mid-column spherules as separate species (i.e., as *E. japonica* and *E. Ritteri* respectively), but because of the variability in presence and extent of that feature in specimens from the Commander Islands, Kamchatka Peninsula, Kurile

Islands, and the Sea of Japan, they were prompted instead to synonymize *E. Ritteri* and *E. japonica*. Sanamyan and Sanamyan (1998) did not report sex allocation for their specimens, but indicated that those with and without the spherules were not significantly different anatomically. The variability in features that they report is characteristic of the Japanese species (Sanamyan and Sanamyan 1998, 2008, 2010) but is not seen in the specimens from Alaska, at least from Adak Island eastward. Based on the differences (described above) between Japanese and Alaskan individuals in sex allocation, characteristics of the brooding groove, and presence of mid-column spherules, we opt to maintain *E. japonica* and *E. Ritteri* as separate species. An intermediate zone of sympatry and/or hybridization may exist somewhere among the Kurile Islands, Kamchatka Peninsula, or Commander Islands.

Furthermore, if rafting is an important mode of long-distance dispersal, as is often assumed for benthic species with non-pelagic development (reviewed by Thiel and Gutow 2005), ocean currents could provide some degree of isolation for the Alaskan individuals from those in Japan and the Kurile and Commander Islands. The Alaskan Stream is a strong westward current which flows along the coast of the Gulf of Alaska and the southern shores of the Aleutian Islands, maximally reaching about 170° E longitude (Favorite 1965). The relatively weak eastward flow on the north side of the Aleutian Islands (Aleutian North Slope Current) primarily originates from waters of the Alaskan Stream moving north through passes between Aleutian Islands (Reed and Stabeno 1994, Chen and Firing 2006).

Chapter 5: Evolution of Brooding in *Epiactis*

Introduction

Reproduction within sea anemones (Actiniaria) is nearly as variable as it is across the rest of the phylum Cnidaria, but the most common scheme is free spawning coupled with pelagic development of larvae (Chia 1976). Among varied modes of development, brooding offspring in or on the body of a parent until it reaches the juvenile stage (i.e. formation of mouth, at least one cycle of tentacles, and pedal disk or physa) is relatively uncommon (Chia 1976). Brooding in sea anemones occurs in internal and external modes: internally brooded offspring are simply retained within the gastrovascular cavity of the parent and released as juvenile sea anemones, whereas externally brooded offspring leave the parent's gastrovascular cavity (or are expelled) but by some means become adhered to or held against the external surface of the parent (Figure 22). While either mode may include or bypass a larval stage, our definition excludes species in which offspring are held within the gastrovascular cavity but released to develop as free swimming pelagic larvae (i.e. larviparity). Larviparity would not be considered brooding by our definition, but internal brooding may evolve in some instances via extended larviparity (i.e. a transition to viviparity). Our usage differs from the somewhat less

restricted terminology in the literature for other cnidarian groups (e.g. corals), in which offspring retained for almost any amount of time and to any developmental stage are frequently referred to as brooders (e.g. “eggs are retained... for up to a few days” Garrabou 1999, p. 202).

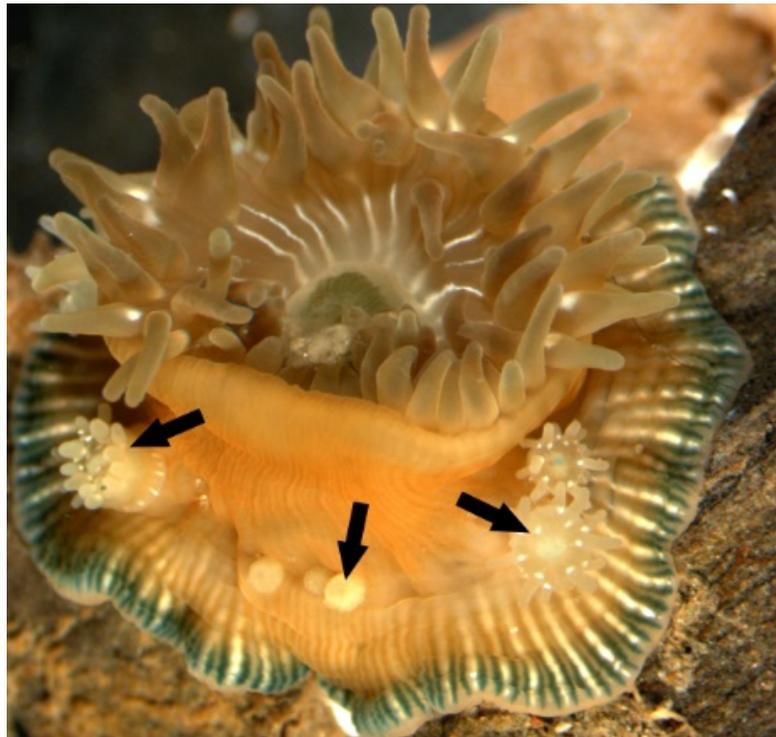


Figure 22 *Epiactis prolifera* with externally brooded offspring (arrow).

Brooding may have ecologically important implications for aspects of reproduction, population dynamics, and development such as dispersal potential, offspring survival, and brood size. Correlates of brooding such as small adult body size and

hermaphroditism are commonly observed in molluscs, echinoderms, and other marine invertebrates (see Ghiselin 1969, Strathmann and Strathmann 1982 and references therein, Strathmann et al. 1984, Byrne 1996, Fernández et al. 2006). Whether such correlations occur in sea anemones is unclear, since closely related brooding species can be dissimilar in size and sex allocation (e.g. *Epiactis prolifera* Verrill 1869a is a small hermaphroditic species but *Epiactis lisbethae* Fautin and Chia 1986 is large and gonochoric).

Monophyly of Epiactis (Actinioidea, Actiniidae)

Epiactis is a broadly distributed genus of sea anemone (Figure 23). Many *Epiactis* species are easily accessible in the rocky intertidal zone, common in their habitat, and variable in color. The genus is characterized by a lack of specialized or elaborate features (e.g., simple tentacles, smooth column, usual (hexamerous) arrangement of mesenteries and tentacles, etc. (e.g., Stephenson 1922, Carlgren 1927, Parry 1951, Hand 1955)) but is notable for the large proportion (11 out of 16) of currently valid species that brood offspring. However, because not all *Epiactis* species brood, and those that do employ diverse modes, the genus as currently understood lacks a synapomorphy. Species of *Epiactis* exhibit a striking array of other reproductive traits including different patterns of sex allocation, allochrony in reproduction, and sexual reproduction that includes uniparental and biparental modes. Among *Epiactis* species found in the north Pacific, internal and external brooding occur along with gonochory and hermaphroditism in all possible combinations (Table 4).

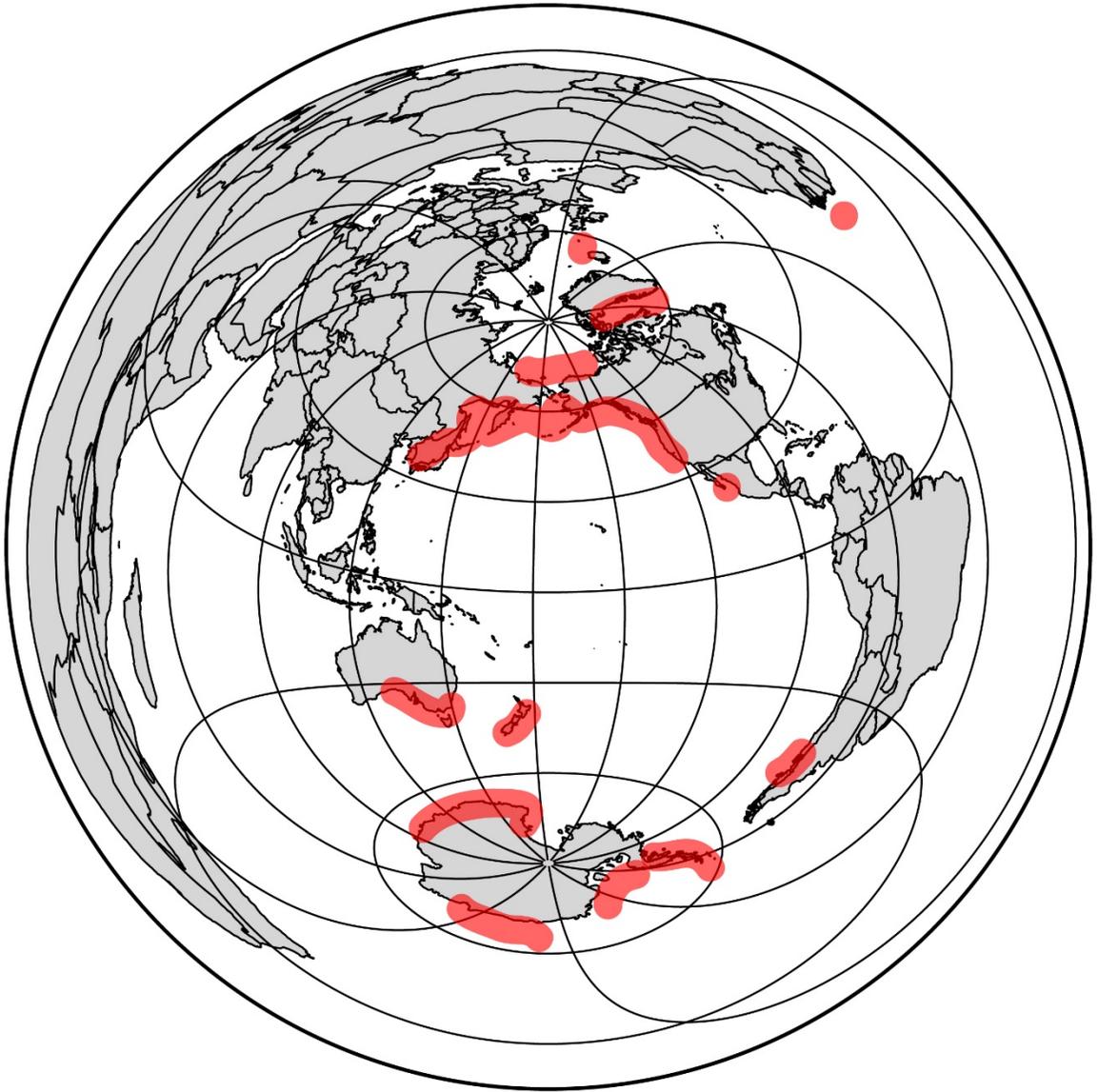


Figure 23 Shaded areas indicate the approximate global distribution of all *Epiactis* species (based on data compiled by Fautin 2015 but excluding *Urticina (Epiactis) fecunda*).

Status of Cnidopus Carlgren 1934

The taxonomic history of *Epiactis* has been complicated by the reassignment of two North Pacific species to *Cnidopus* (Carlgren 1934). *Cnidopus* contained three species at its maximum and was created to account for the holotrich-dense protuberances at and near the limbus of *E. Ritteri* Torrey, 1902. Carlgren (1950) also later transferred *E. japonica* (Verrill 1869b) and *Actinia veratra* (Drayton in Dana 1846) to *Cnidopus* citing similar features. Fautin and Chia (1986) synonymized *Cnidopus* with *Epiactis*, in part, based on the discovery of holotrichs near the limbus in *E. prolifera* and on specimens of what Hand and Dunn (1974) identified as *C. Ritteri* but which has since been described as *Epiactis handi* Larson and Daly, 2015. Edmands and Fautin (1991) later moved *C. veratra* to *Aulactinia* Agassiz in Verrill, 1864, but *C. japonicus* (Verrill 1869b) has not yet been evaluated for generic placement. Thus the synonymy of the genera remains contentious, with some authors continuing to refer to *C. japonicus* and *C. Ritteri* (e.g., Sanamyan and Sanamyan 1998, Ishimura and Nishihira 2002) and others favoring *E. japonica* and *E. Ritteri* (e.g., Fautin and Chia 1986, Edmands 1995, Larson and Daly 2015).

Species	Reproduction	Sex allocation	Brood location	Range	Source
<i>E. arctica</i>	-	Gonochoric	External	AR	Verrill 1868, 1899
<i>E. australiensis</i>	-	-	-	AU	Carlgren 1950
<i>E. fernaldi</i>	-	Hermaphroditic	Internal	NP	Fautin & Chia 1986
<i>E. georgiana</i>		Gonochoric + rare hermaphrodites	External	AN	Rodríguez et al. 2012b
<i>E. handi</i>	-	Gonochoric	Internal	NP	Hand & Dunn 1974
<i>E. incerta</i>	-	Gonochoric†	-	AR	Carlgren 1921
<i>E. irregularis</i>	-	-	-	NP	Carlgren 1951
<i>E. japonica</i>	Seasonal	Hermaphroditic	External	NP	Uchida 1934, Uchida & Iwata 1954
<i>E. lewisi</i>	-	-	External	AR	Carlgren 1940
<i>E. lisbethae</i>	Seasonal	Gonochoric	External	NP	Fautin & Chia 1986
<i>E. marsupialis</i>	-	Gonochoric	External	AR	Carlgren 1893
<i>E. neozelandica</i>	-	-	-	AU	Stephenson 1918
<i>E. nordmanni</i>	-	Gonochoric†	-	AR	Carlgren 1921
<i>E. prolifera</i>	Continuous	Gynodioecious	External	NP	Dunn 1975a, 1975b; Verrill 1869a
<i>E. ritteri</i>	Seasonal	Gonochoric	External	NP	Larson & Daly 2015
<i>E. thompsoni</i>	Continuous*	Gonochoric*	Internal	AU	Stuckey 1909b
<i>E. vincentina</i>	-	Gonochoric†	-	AT	Carlgren 1939

Table 4 *Epiactis* species and reproductive traits. AR=arctic, AU=Austral, NP=North Pacific, AN=Antarctic, AT=Atlantic. *=Personal Observation (PL), †=conclusion based on single specimen, -=Information Not Reported

Features Associated with Brooding

The intrageneric diversity in reproductive characteristics among closely related individuals provides a convenient opportunity to evaluate hypotheses in the evolution of, and evolutionary transitions in, morphological and behavioral characters associated with brooding. Chia (1976 p. 267) hypothesized that external and internal brooders were more closely related to oviparous and larviparous spawners, respectively, than they were to one another. Previous phylogenetic work in *Epiactis* has also separated external and internal brooders (Edmands 1996) although this work was relatively restricted in taxonomic and geographic scope, evaluating allozyme data for seven species from the North American Pacific coast: two externally (*E. lisbethae* Fautin and Chia, 1986 and *E. prolifera*) and three internally brooding species (*Aulactinia incubans* Dunn et al. 1980, *Epiactis fernaldi* Fautin and Chia 1986 and *E. handi*); and two non-brooding species (*Anthopleura elegantissima* (Brandt 1835) and *Metridium senile* (Linnaeus 1761)). None of Edmands' (1996) phylogenetic reconstructions recovered a monophyletic *Epiactis*, several did not recover a monophyletic ingroup, and *A. elegantissima* was always recovered as sister to the externally brooding species of *Epiactis*.

Results of previous investigations, the disjunct distribution of *Epiactis*, diversity in reproductive strategies, and the lack of synapomorphies call into question the monophyly of the genus, and therefore the relationship among instances of brooding. Here we revisit Edmands' (1996) work using nucleotide sequence data and expand the geographic and taxonomic range for a more thorough sampling of brooding taxa across *Epiactis* and Actiniidae. Our molecular phylogeny of *Epiactis* species includes brooding and non-

brooding members of Actiniidae, which contains 44 genera and at least 200 species (Daly et al. 2007), to investigate the monophyly of *Epiactis* and the evolutionary relationships of characters associated with reproduction. We evaluate the validity of *Cnidopus* and test for character correlation between the brooding offspring and the hermaphroditic condition in the context of our phylogenetic inference.

Materials and Methods

Sample and Data Collection

New specimens were collected at low tide from intertidal areas of sampling sites in New Zealand, Japan, Alaska, British Columbia, Washington, and California between 2010 and 2014 (Table 5). Individuals were removed from their substrate with a blunt knife or a spatula. Live animals were brought back to a lab for processing: a tissue sample from the edge of the pedal disk was preserved in 90–100% ethanol while the rest of the specimen was fixed in 10% sea water-buffered formalin. Anatomical vouchers, when possible, have been deposited at the American Museum of Natural History (Table 6).

Whole genomic DNA was extracted from ethanol-preserved pieces of limbus using the DNeasy Blood and Tissue extraction kit (Qiagen) following manufacturer's protocols. For 76 individuals representing 38 species, we used primers to amplify 3 mitochondrial genes (12S, 16S, and CO3) and 3 nuclear loci (18S, 28S, and ITS) by PCR (see Table 6). PCR products were sequenced via Sanger Sequencing by Beckman Genomics (Danvers, MA). New sequences generated for this study are available from GenBank (accession numbers: 12S, KT851988–KT852049; 16S, KT852050–KT852117; 18S, KD852118–

KT852179; ITS, KT852180–KT852244; 28S KT852245–KT852268; CO3, KT852269–KT852334 and see table 6). The taxon sample includes multiple representatives of genera whose members brood and/or have been affiliated with *Epiactis* and several outgroup taxa with varying amounts of phylogenetic distance to the ingroup. Raw sequence data from previously published studies were included in the analysis (Table 6).

Genus	Species	Locality
<i>Epiactis</i>	<i>prolifera</i>	California and Washington, USA; Vancouver Island and Haida Gwaii, British Columbia, Canada
<i>Epiactis</i>	<i>handi</i>	Haida Gwaii, British Columbia, Canada
<i>Epiactis</i>	<i>ritteri</i>	Kodiak Island and Adak Island, Alaska, USA
<i>Epiactis</i>	<i>japonica</i>	Hokkaido, Japan
<i>Epiactis</i>	<i>lisbethae</i>	Washington, USA
<i>Epiactis</i>	<i>thompsoni</i>	North and South Islands, New Zealand
<i>Aulactinia</i>	<i>vancouverensis</i>	Kodiak Island, Alaska, USA
<i>Urticina</i>	<i>grebelnyi</i>	Popof Island, Alaska, USA
<i>Urticina</i>	<i>crassicornis</i>	Adak Island, Alaska, USA
<i>Actinia</i>	<i>tenebrosa</i>	New Zealand
<i>Oulactis</i>	<i>muscosa</i>	New Zealand
<i>Isactinia</i>	<i>olivacea</i>	New Zealand
<i>Anthopleura</i>	<i>rosea</i>	New Zealand
<i>Charisea</i>	<i>saxicola</i>	Kodiak Island, Alaska, USA
<i>Cricophorus</i>	<i>nutrix</i>	New Zealand

Table 5 New specimens collected for this study and their general localities.

Phylogenetic analysis

Sequences were aligned in Muscle v. 3.8.425 (Edgar 2004) as implemented in Geneious v. 7.1.5 (Biomatters) using default parameters. Matrices were analyzed under Maximum Likelihood (ML) and Maximum Parsimony (MP) optimality criteria. For ML analyses, matrix partition scheme (starting with all markers as separate partitions and CO3

partitioned further by codon) and best model compatible with RAxML (Stamatakis 2014) were implemented as determined under the corrected Akaike Information Criterion by PartitionFinder v. 1.1.1 (Lanfear 2012) for nuclear, mitochondrial, and combined matrices. Matrices consisting of mitochondrial, nuclear, and all loci were analyzed separately for MP analyses. See Table 7 for details about the matrices.

The MP analysis implemented in TNT v. 1.1 (Goloboff 2008) on the complete data set consisted of a driven search at level 99 and included 1000 initial search replicates. Search strategies combined sectorial search, ratchet, drifting, and tree fusing in default parameters. 1000 replicates of jackknife resampling were performed on the strict consensus tree to assess clade support. The ML analyses implemented in RAxML v. 8.1.16 consisted of 20 independent searches followed by 10,000 rounds of bootstrapping on the best tree.

Character analysis

Character state data were collected from published descriptions and personal observations. Ancestral state reconstruction for brooding mode and sex allocation was implemented on the best ML tree topology in Mesquite v. 3.02 (Maddison and Maddison 2011) via Maximum Parsimony (with unordered states) and Maximum Likelihood (Marginal probability reconstruction MK1 Model). Tests for character correlation between sex allocation and brooding were carried out in Mesquite using the Pagel94 test of independent evolution, under various character coding schemes. The test requires binary characters and complete data. The binary character states used for brooding mode in the three separate analyses were brooding/not, external brooding/not, and internal

brooding/not; and for sex allocation was hermaphroditic/gonochoric (see discussion for rationale). Taxa with missing data were excluded from the correlation analysis. P-values for likelihood ratio tests were estimated based on 1000 simulation replicates.

Results

Phylogenetic analysis

PartitionFinder results favored the GTR+I+G substitution model and matrices with 6 partitions (by locus, except that CO3 codon position 1 was grouped with the 16S partition). An additional PartitionFinder run using the AIC returned identical results in terms of model selection and partition scheme. The best scoring tree of 20 ML runs on the full data set is shown in Figure 24. Alignments and trees are available from treebase (accession URL is <http://purl.org/phylo/treebase/phyloids/study/TB2:S18336>). In the 20 runs, *Epiactis* species from the North Pacific form an exclusive clade and are always reciprocally monophyletic at the species level, except for *E. handi*, among which the sole representative of *E. fernaldi* is nested, and *E. lisbethae*, which is monophyletic with respect to *E. prolifera* in only 5 of the 20 ML runs. *Epiactis* species from the Southern hemisphere do not cluster with those from the north Pacific, but instead form a paraphyletic grade with respect to southern hemisphere species of *Aulactinia* Agassiz in Verrill 1864. Worldwide, *Epiactis* species do not constitute a monophyletic group, but the type species (*E. prolifera*) is a member of a well supported (99 bootstrap, 97

Table 6 Caption: Taxa and sequences included in this analysis. New sequences generated for this study are in bold.

Table 6

Family	Species	ID	Location	12S	16S	18S	28S	CO3	ITS
Actiniidae	<i>Actinia fragacea</i>	AFRA	NA	EU190714.1	EU190756.1	EU190845.1	KJ483085.1	GU473334.1	KT852191
	<i>Actinia tenebrosa</i>	TEN	KUNHM	KT852045	KT852111	KT852174	----	KT852330	KT852239
	<i>Anthopleura artemisia</i>	KOD1	NA	KT852015	KT852081	KT852148	----	KT852300	KT852210
	<i>Anthopleura atodai</i>	AAT	NA	KT851993	KT852055	KT852123	KT852247	KT852275	KT852185
	<i>Anthopleura elegantissima</i>	AELE	NA	----	----	----	KT852248	----	----
	<i>Anthopleura handi</i>	HAN	NA	KT852013	KT852079	KT852146	----	KT852298	KT852208
	<i>Anthopleura rosea</i>	RUB	NA	KT852039	KT852104	KT852168	----	KT852324	KT852232
	<i>Aulactinia incubans</i>	INC	NA	KT852014	KT852080	KT852147	KT852256	KT852299	KT852209
	<i>Aulactinia marplatensis</i>	AMAR	AMNH	KT851999	KT852061	KT852129	KT852249	KT852281	KT852192
	<i>Aulactinia sp.</i>	P7	AMNH	----	----	KT852164	----	----	----
	<i>Aulactinia stella</i>	TELL	AMNH	KT852044	KT852110	KT852173	KT852263	KT852329	KT852238
	<i>Aulactinia vancouverensis</i>	KOD5	AMNH	KT852019	KT852085	KT852151	----	KT852305	KT852214
	<i>Aulactinia veratra</i>	AVER	AMNH	KT852001	KT852063	KT852131	----	KT852283	KT852194
	<i>Bunodactis reynaudi</i>	SAF409	NA	KT852041	KT852106	KT852170	KT852260	KT852326	KT852234
	<i>Bunodactis verrucosa</i>	BVER	AMNH	EU190723.1	EU190766.1	EU190854.1	KT852250	FJ489484.1	KT852195
	<i>Epiactis australiensis</i>	AUS2	MV	KT852000	KT852062	KT852130	----	KT852282	KT852193
	<i>Epiactis fernaldi</i>	EFER	NA	KT852005	KT852068	KT852136	KT852252	KT852288	KT852201
	<i>Epiactis georgiana</i>	EPIG	NA	KT852007	KT852070	KT852138	KT852254	KT852290	KT852203
	<i>Epiactis handi</i>	1EC6	AMNH	KT851988	KT852050	KT852118	KT852245	KT852269	KT852180
	<i>Epiactis handi</i>	3EC2	AMNH	KT851990	KT852052	KT852120	KT852268	KT852271	KT852182
<i>Epiactis handi</i>	CB1	AMNH	KT852002	KT852064	KT852132	KT852251	KT852284	KT852196	

Continued

Table 6 continued

<i>Epiactis handi</i>	CB1	AMNH	KT852002	KT852064	KT852132	KT852251	KT852284	KT852196
<i>Epiactis handi</i>	ERIT	AMNH	KT852009	KT852072	KT852140	KT852255	KT852292	KT852205
<i>Epiactis handi</i>	ST8	AMNH	KT852043	KT852109	KT852172	KT852262	KT852328	KT852237
<i>Epiactis handi</i>	TH10	AMNH	KT852046	KT852112	KT852175	KT852264	KT852331	KT852240
<i>Epiactis handi</i>	YP8	AMNH	KT852049	KT852117	KT852179	KT852267	KT852334	KT852244
<i>Epiactis japonica</i>	A1	AMNH	KT851991	KT852053	KT852121	----	KT852272	KT852183
<i>Epiactis japonica</i>	A3	AMNH	KT851992	KT852054	KT852122	----	KT852273	KT852184
<i>Epiactis japonica</i>	MS1J	AMNH	KT852025	KT852090	KT852155	----	KT852310	----
<i>Epiactis japonica</i>	MS2G	AMNH	KT852026	KT852091	KT852156	----	KT852311	KT852220
<i>Epiactis japonica</i>	MU13	AMNH	KT852027	KT852092	KT852157	----	KT852312	KT852221
<i>Epiactis japonica</i>	MU4	AMNH	KT852028	----	KT852158	----	KT852313	KT852222
<i>Epiactis japonica</i>	N15	AMNH	KT852029	KT852093	KT852159	----	KT852302	KT852223
<i>Epiactis japonica</i>	N4	AMNH	KT852030	KT852094	KT852160	----	KT852314	KT852224
<i>Epiactis japonica</i>	O2A C	AMNH	KT852031	KT852095	----	----	KT852315	KT852225
<i>Epiactis japonica</i>	O2B C	AMNH	KT852032	KT852096	KT852161	----	KT852316	----
<i>Epiactis japonica</i>	SA5	AMNH	KT852040	KT852105	KT852169	----	KT852325	KT852233
<i>Epiactis japonica</i>	SB7	AMNH	KT852042	KT852108	KT852171	----	KT852327	KT852236
<i>Epiactis japonica</i>	US27	AMNH	KT852047	KT852115	KT852177	----	KT852274	KT852242
<i>Epiactis japonica</i>	US8	AMNH	KT852048	KT852116	KT852178	----	KT852333	KT852243
<i>Epiactis lisbethae</i>	ELIS	NA	KT852006	KT852069	KT852137	KT852253	KT852289	KT852202
<i>Epiactis lisbethae</i>	RAN	NA	----	KT852103	KT852167	KT852259	KT852323	KT852231

Continued

Table 6 continued

<i>Epiactis prolifera</i>	2EC8	AMNH	KT851989	KT852051	KT852119	KT852246	KT852270	KT852181
<i>Epiactis prolifera</i>	cjapu	AMNH	KT852003	KT852065	KT852133	----	KT852285	KT852197
<i>Epiactis prolifera</i>	epr1	AMNH	KT852008	KT852071	KT852139	----	KT852291	KT852204
<i>Epiactis prolifera</i>	PIL1	AMNH	KT852035	KT852099	KT852165	----	KT852319	KT852228
<i>Epiactis prolifera</i>	pp14	AMNH	KT852036	KT852100	KT852166	----	KT852320	----
<i>Epiactis prolifera</i>	pp6	AMNH	KT852037	KT852101	----	----	KT852321	KT852229
<i>Epiactis prolifera</i>	pp7	AMNH	KT852038	KT852102	----	----	KT852322	KT852230
<i>Epiactis ritteri</i>	ADK11	AMNH	KT851994	KT852056	KT852124	----	KT852276	KT852186
<i>Epiactis ritteri</i>	ADK4	AMNH	KT851995	KT852057	KT852125	----	KT852277	KT852187
<i>Epiactis ritteri</i>	ADK5	AMNH	KT851996	KT852058	KT852126	----	KT852278	KT852188
<i>Epiactis ritteri</i>	ADK9	AMNH	KT851998	KT852060	KT852128	----	KT852280	KT852190
<i>Epiactis ritteri</i>	KOD10	AMNH	KT852016	KT852082	KT852149	----	KT852301	KT852211
<i>Epiactis ritteri</i>	KOD11	AMNH	KT852017	KT852083	----	----	KT852303	KT852212
<i>Epiactis ritteri</i>	KOD2	AMNH	KT852018	KT852084	KT852150	----	KT852304	KT852213
<i>Epiactis ritteri</i>	KOD7	AMNH	KT852021	KT852087	KT852153	----	KT852307	KT852216
<i>Epiactis ritteri</i>	KOD9	AMNH	KT852022	KT852088	KT852154	----	KT852308	KT852217
<i>Epiactis thompsoni</i>	ETH1	AMNH	KT852010	KT852073	KT852141	----	KT852293	----
<i>Epiactis thompsoni</i>	ETH2	AMNH	KT852011	KT852074	KT852142	----	KT852294	KT852206
<i>Epiactis thompsoni</i>	ETH8	AMNH	----	KT852075	----	----	KT852295	----
<i>Glyphoperidium bursa</i>	GLY	AMNH	KJ482923.1	KT852076	KT852143	----	KJ482982.1	----
<i>Gyractis sesere</i>	GSE	NA	KT852012	KT852078	KT852145	----	KT852297	----
<i>Isactinia olivacea</i>	Gra	AMNH	----	KT852077	KT852144	----	KT852296	KT852207

Continued

Table 6 Continued

	<i>Oulactis muscosa</i>	OMUS	AMNH	KT852033	KT852097	KT852162	----	KT852317	KT852226
	<i>Urticina coriacea</i>	U2	AMNH	GU473282.1	KT852114	KT852176	KT852265	GU473351.1	----
	<i>Urticina crassicornis</i>	ADK6	AMNH	KT851997	KT852059	KT852127	----	KT852279	KT852189
	<i>Urticina fecunda</i>	Efec1	CMNI	KT852004	KT852067	KT852135	----	KT852287	KT852200
	<i>Urticina grebelnyli</i>	P1	AMNH	KT852034	KT852098	KT852163	----	KT852318	KT852227
Condylanthidae	<i>Charisea saxicola</i>	KOD6	AMNH	KT852020	KT852086	KT852152	----	KT852306	KT852215
Edwardsiidae	<i>Edwardsia timida</i>	TIM	KUNHM	GU473281.1	KT852113	GU473315.1	KT852265	KT852332	KT852241
Hormathiidae	<i>Cricophorus nutrix</i>	Crico	AMNH	----	KT852066	KT852134	----	KT852286	KT852199
Metridiidae	<i>Metridium senile</i>	MET	KUNHM	KT852024	EU190786.1	----	KT852258	----	KT852219
	<i>Met. senile fimbriatum</i>	MEFI	KUNHM	KT852023	KT852089	JF832988.1	KT852257	KT852309	KT852218
Sagartiidae	<i>Cereus pedunculatus</i>	CPE	KUNHM	----	EU190767.1	EU190855.1	----	----	KT852198
	<i>Sagartia troglodytes</i>	SAT	KUNHM	EU190746.1	KT852107	EU190872.1	KT852261	FJ489499.1	KT852235

jackknife) monophyletic, geographically restricted clade (Figure 24) referred to hereafter as *Epiactis sensu stricto*. A minor clade of *Aulactinia* species (also a polyphyletic genus in our results) are recovered as a sister group to *Epiactis sensu stricto*.

The results of independently analyzed mitochondrial (Figure 25) and nuclear data (Figure 26) differed from the combined analysis and from each other to varying extent in terms of topology and support values. Mitochondrial data gave more support to deeper nodes. Relationships within *Epiactis sensu stricto* and the group itself had low support by mitochondrial markers. Mitochondrial data also placed (bootstrap support of 19) the clade of southern hemisphere *Epiactis* spp. as sister to the clade *Epiactis sensu stricto* + *Aulactinia* spp. The nuclear matrix yielded a slightly different topology from the combined and mitochondrial data in that *E. georgiana* Carlgren, 1927 nested within a paraphyletic grade comprised of species of *Urticina* Ehrenberg, 1834 while *E. thompsoni* (Coughtrey 1875) and *E. australiensis* Carlgren, 1950 remained in the clade of Southern Hemisphere species. Nuclear data resolved the species nodes more effectively within *Epiactis sensu stricto*.

The concatenated alignment contained 1304 parsimony informative characters (12S, 78; 16S, 56; 18S, 191; 28S, 310; CO3, 139; ITS, 529). For the concatenated matrix, 180 most parsimonious trees of length 5740 were found. The strict consensus (Figure 27) of the 180 trees has a CI = 0.597 and a RI = 0.660 and includes a monophyletic clade of *Epiactis* species from the North Pacific, though these reciprocally monophyletic groups had poor jackknife support. MP strict consensus topological results differ from ML in

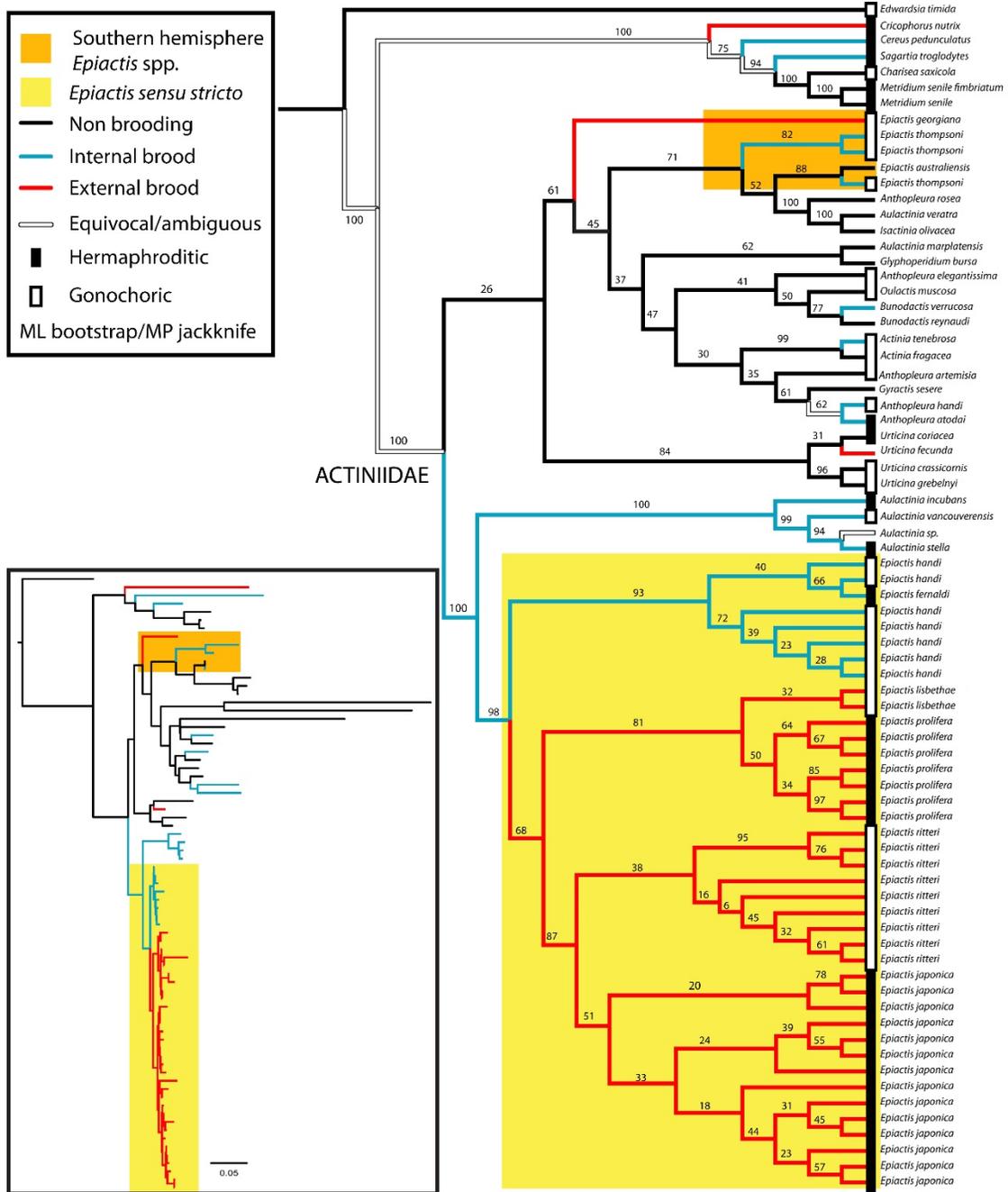


Figure 24 Best scoring tree of 20 maximum likelihood runs using complete data set. Branch lengths set equal for clarity. Colored and hollow branches correspond with Parsimony ancestral character state reconstruction for brooding mode. Inset: same tree with proportional branch lengths. *Epiactis sensu stricto* (yellow box) are exclusively northern hemisphere species.

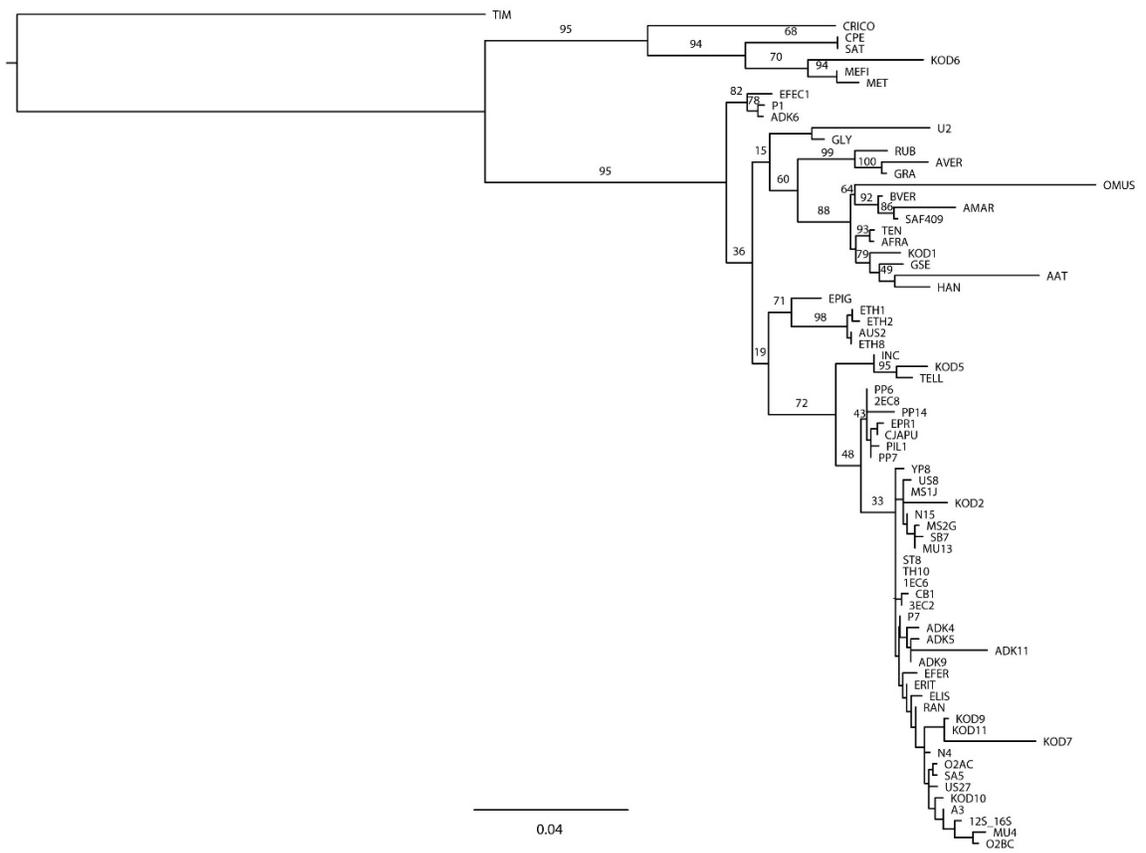


Figure 25 Maximum Likelihood results from mitochondrial matrix. Some support values moved or removed for clarity. See table 6 for terminal species codes.

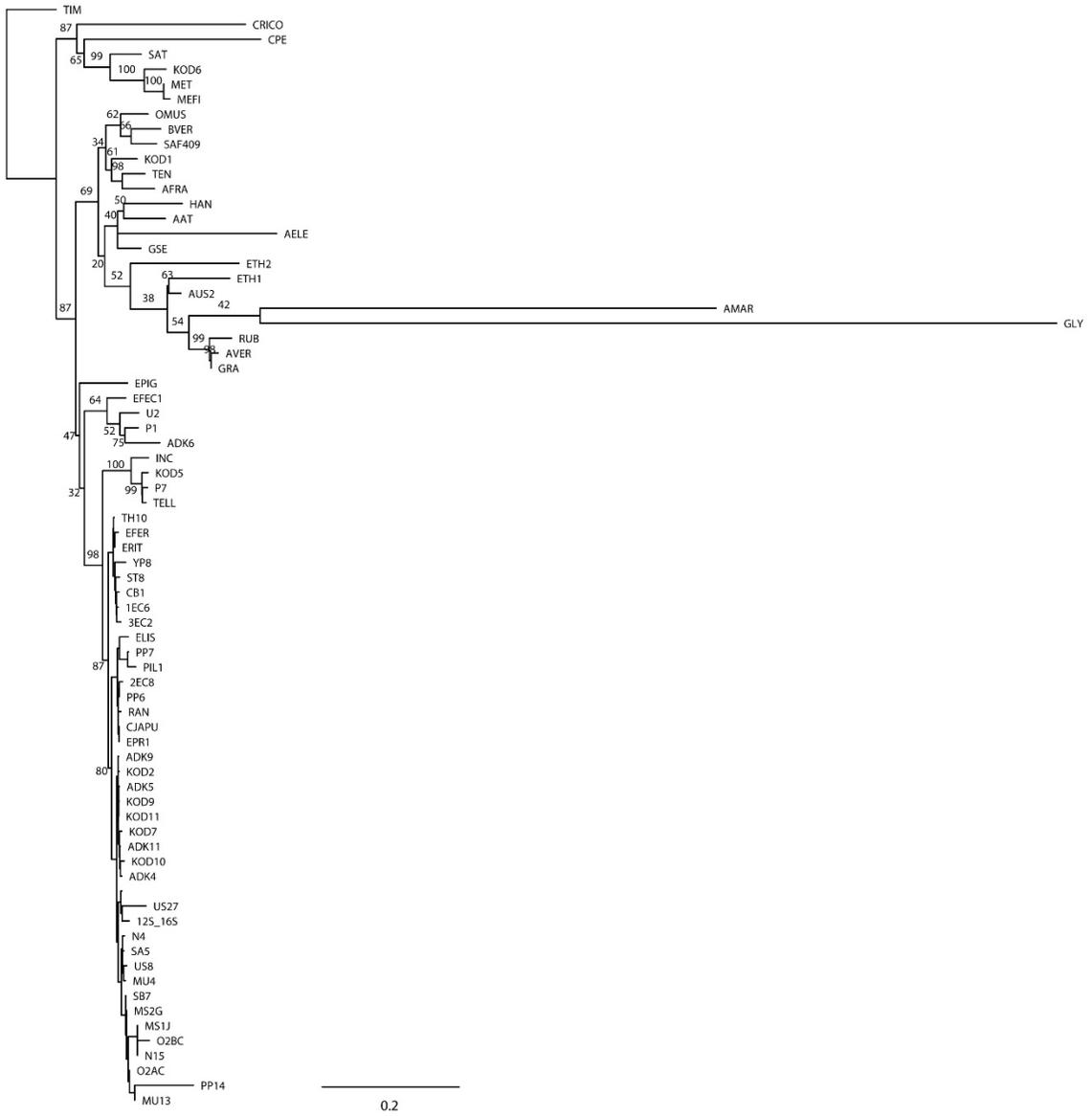


Figure 26 Maximum Likelihood results for nuclear matrix. Some support values moved or removed for clarity. See table 6 for terminal species codes.

Figure 27 Strict consensus of Maximum Parsimony results from complete data matrix.
Nodes with <50% jackknife support are collapsed. Red branches-external brooding
species, blue branches-internally brooding species.

that *Epiactis georgiana* is recovered basal to the *Aulactinia* clade that is sister to *Epiactis sensu stricto*, and species of *Urticina* form a paraphyletic grade at the base of Actiniidae. Multiple rearrangements occur among taxa within the major clade that does not contain *Epiactis s.s.*

Character analysis

Brooding taxa are scattered throughout the tree, and ancestral character state reconstructions indicate multiple independent gains of brooding and no losses. Parsimony (Figure 24) and likelihood (Figure 28) character reconstruction methods for brooding modes yield similar results: external brooding in *Epiactis sensu stricto* has evolved as a derivation from an internally brooding ancestor. External brooding appears independently in *Urticina fecunda* (Verrill 1899). Because there are internally brooding (and viviparous) species in the closely related genus *Cribrinopsis* Carlgren 1921 and because sampling was incomplete for *Urticina*, the nature of this transition is less clear and requires both additional taxonomic and phylogenetic study to resolve. Internal brooding is apparently more labile than external brooding as evidenced by the multiple independent origins of that character (Figure 24). Sex allocation is labile even within *Epiactis sensu stricto*, with various forms of hermaphroditism arising independently multiple times (Figure 28).

Figure 28 Maximum likelihood topology with parsimony (branch color) and Likelihood (pie chart) ancestral state estimation for sex allocation mode and likelihood (pie chart) ancestral state estimation for brooding mode (see Figure 24 for parsimony ancestral state estimation for brooding).

In the most general test (for any effect) of the character correlation analyses, the 8 parameter model (correlated character evolution) was not an improvement over the 4 parameter model (independent character evolution), having a log likelihood difference of 3.21, $p = 0.091$. Thus we find no evidence for correlation of brooding (in general) and sexual allocation system in the taxa included in the analysis. Internal and external brooding specifically also do not correlate with sex allocation (log likelihood difference of 2.88, $p = 0.078$ and log likelihood difference of 3.29, $p = 0.10$, respectively).

Locus	Shortest	Longest	Aligned
12S	539	830	858
16S	352	481	509
18S	1120	1789	1956
28S	540	3256	3397
CO3	447	674	679
ITS	511	768	1071

Table 7 Sequence and matrix lengths for each locus.

Discussion

Many reproductive features, including brooding, are complex characters not easily dissected into constituent parts. This is especially true in the case of the focal species, for which many details of reproductive biology are unknown. The terms ‘internal’ and ‘external’ are coarse categories of behavior and anatomy and are not meant to imply homology: indeed we sought here, in part, to test the homology of brooding implied by current classification. Chia (1976) described reproductive behaviors in comparatively

complex terms addressing fertilization, larval habit, and larval feeding. Nonetheless, his categories included two groups that closely correspond with our internal brooding and external brooding modes, “Oviparous-brooding-lecithotrophic” and “viviparous,” respectively.

“Oviparous-brooding-lecithotrophic” describes reproduction in species like *Epiactis prolifera*, in which zygotes or early embryos are released from the gastrovascular cavity and then attach to the ectoderm of the column for development. This category could be named more generally (removing “oviparous”) to account for species which release larvae rather than eggs, but still externally brood to the juvenile stage (an unknown phenomenon in 1976: see Larson et al. (2012)). Chia’s (1976) “viviparous” group is nearly equivalent with what we term “internal brooders,” (i.e. those that retain offspring within the adult gastrovascular cavity and release juvenile sea anemones), although we also consider the asexually produced juveniles released from e.g., species of Boloceroididae (not included in this analysis) to be internally brooded whereas Chia (1976) placed those in the “budding” behavior category. The asexual nature of internally brooded boloceroidids matches that of species (e.g. *Actinia equina* (Linnaeus 1758)) in Chia’s viviparous group (Carter and Thorp 1979, Orr et al. 1982). That internal brooding encompasses asexually produced and sexually produced forms may in part explain its apparent evolutionary lability relative to external brooding (Figure 24), for which only sexually-produced offspring have been reported when studied.

Implications of phylogeny

Though the combined dataset and data subsets differ slightly in their topologies, we preferred *a priori* to consider the data in its entirety as the most robust of the analyses (Kluge 1998). *A posteriori*, combined data also gives the most geographically and taxonomically coherent result. Therefore we will discuss our results and frame our conclusions based on the combined data results.

Status of Epiactis.

All of our analyses recovered *Epiactis* as a polyphyletic assemblage. The type species, *Epiactis prolifera* is always found within a monophyletic group of species of *Epiactis* from the north Pacific. This clade constitutes *Epiactis sensu stricto*. This result is contrary to the previous findings of Edmands (1996) who analyzed alleles for 20 allozyme loci in distance and parsimony frameworks and found *Epiactis* species from the North Pacific split from one another by *Anthopleura elegantissima*. In our results, based on nucleotide sequence data, no species of *Anthopleura* is closely related to any species of *Epiactis sensu stricto*.

We find that the *Epiactis* species from Antarctica, New Zealand, and Australia cluster in a clade with other southern hemisphere species (ML results) or are spread more broadly throughout the tree (MP results). This highlights the need for further work in the southern group. Thorough taxonomic sampling of the southern hemisphere species and any northern relatives and more informative markers are needed to help resolve the issues of

relatively long branches and weaker support for confident assessment of the relationships and character evolution in that clade, respectively. In addition, the placement of *Epiactis australiensis* within the *Epiactis thompsoni* group suggests that taxonomic work in this group is yet incomplete and may involve still more species discovery. The finding that the southern hemisphere *Epiactis* species are closely related with southern hemisphere *Aulactinia* species mirrors the relationship of those genera in the northern hemisphere and suggests that geographic distance may have greater implications for taxonomy than initially recognized, especially in brooding or other species with lowered dispersal potential. Since samples of *Aulactinia capitata* Agassiz in Verrill 1864, the type species, were not available for this study, we cannot suggest any specific revisions to the genus *Aulactinia*, but recommend the group for further study in a more focused analysis. Samples preserved for genetic analysis could not be acquired for several *Epiactis* species. Particularly desirable are samples of the arctic brooding species *E. lewisi* Carlgren 1940, *E. arctica* (Verrill 1868), and *E. marsupialis* Carlgren 1901 and the subantarctic *Bunodactis (Epiactis) mortenseni* (Carlgren 1924). These are infrequently collected (or perhaps infrequently identified), primarily deep water (except *B. mortenseni*) species that brood externally and use column modifications in the form of shallow pits to retain brooded offspring. The latter feature is not found in the *Epiactis* species in our analysis but is found in *Urticina fecunda* (Larson et al. 2012). The recognition of *E. ritteri* and *E. japonica* as separate species has been challenged (e.g. Sanyaman and Sanyaman 1998), but present results corroborate the finding of their distinctness based on morphological characters (Larson and Daly 2015). The two species

are reciprocally monophyletic in our analyses though these nodes are weakly supported in the combined analysis (Figure 24). The pattern of relationships within this clade is suggestive of a north and westward expansion into Japan by an externally brooding species.

Finally, our results allow us to comment on the position of *Epiactis sensu stricto* within the Actiniaria in general and superfamily Actinioidea more specifically. Previous higher-level phylogenetic studies have consistently placed a representative *Epiactis* species (*E. lisbethae*) among the basal members of the Endomyaria (Rodríguez and Daly 2010, Rodríguez et al. 2012a, Daly et al. 2008, 2010), or even as basal Metridioidea (Rodríguez et al. 2014). Although this basal position is an apparently intuitive result given the lack of specialized features in *Epiactis* species (Rodríguez et al. 2014), our more thoroughly-sampled analyses place *Epiactis* species (including *E. lisbethae*) firmly within the Actinioidea.

Status of Cnidopus.

Because we find the type species of *Cnidopus* (*E. ritteri*) within the clade that also includes *E. prolifera*, the type species of *Epiactis*, we consider *Cnidopus* to be a junior subjective synonym of *Epiactis*. We interpret the differences in diagnoses to reflect errors of omission: every generic description or diagnosis of *Epiactis* (Verrill 1869a, Torrey 1902, Stephenson 1918, 1922, Carlgren 1921, 1927, 1949, Parry 1951, Hand 1955) published before Carlgren's (1934) creation of *Cnidopus* excluded the characters for which *Cnidopus* was erected. These issues highlight the need for a generic diagnosis that

is comprehensive in terms of the features it considers; given the inferred relationships among nominal species of *Epiactis* (Figure 24), this diagnosis should differentiate and exclude species such as those in the southern hemisphere that are demonstrably separate from *Epiactis sensu stricto*. The characters sufficient for such distinction are not present in published accounts, and with the untested relationship of the externally brooding Arctic *Epiactis* species with the North Pacific species, we refrain from a complete overhaul of genus-level taxonomy at this point.

Ecological perspectives on brooding.

Brooding is often assumed to be an adaptive behavior with the primary benefit being increased survival of offspring through parental protection, but with the tradeoffs of reduced fecundity and dispersal (Menge 1975, Chia 1976). Attempts to identify ecological pressures which explain the adaptive significance of brooding in particular are often subsumed within discussions of the more general phenomenon of benthic-lecithotrophic development. Benthic-lecithotrophic development (including brooding) was interpreted to be an adaptive response to harsh living conditions in cold and dark water, and the idea that pelagic development is largely absent from polar and deep water is known as “Thorson’s Rule” (Thorson 1950). The explanations proposed for this apparent pattern often include selection against pelagic planktotrophy due to, e.g., low food availability in the plankton caused by lack of light in the deep or extreme photoperiodic cycles at the poles (Thorson 1936, 1950), and low metabolic rate leading to extended developmental periods in cold water (Hoegh-Guldberg and Pearse 1995) and

thus low survivorship for pelagic offspring. The problems with Thorson's Rule begin with the large number of counterexamples, i.e. pelagic and planktotrophic larvae in cold waters (e.g. Bosch and Pearse 1990) and pre-Thorson observations (reviewed by Young 1994), and include the fact that some cold water habitats (i.e. deep sea and arctic) do not show the same preponderance of brooding species as the Antarctic for many taxonomic groups (Pearse and Lockhart 2004).

Noting exceptions to the rule, namely the persistence of benthic lecithotrophy in the presence of ample planktonic food sources and the highly successful alternative strategies of planktivory and detritivory in polar climates, Chia (1974) refocused the explanation for brooding on the parent, rather than the offspring. Chia (1974) proposed that brooding is a "poor man's game:" with limited energy available for gamete production, an individual should reproduce in the most efficient manner per unit energy cost (i.e. high survival of relatively few offspring via lecithotrophy). Brooding would presumably increase the survivorship of lecithotrophic offspring (and thus the efficiency of this reproductive mode) through the increased levels of protection granted by living in or on the adult.

Empirical evidence for an adaptive explanation to the distribution of brooding species is scarce, however. Pearse et al. (2009) evaluated possible adaptive and non-adaptive explanations for brooding in several Antarctic echinoderm and crustacean systems and concluded that conditions in Antarctica likely promote species diversification (through a species pump caused by repeated glacial cycles or through rare rafting events propelled by the Antarctic Circumpolar Current –the "ACC hypothesis"), especially in those

species with already limited dispersal capabilities such as brooders. The abundance of Antarctic brooders, then, is a phylogeographic phenomenon rather than an adaptive one. In sea anemones, too, brooding in general does not appear to be an adaptation to cold water. Internally brooding species have a pan-latitudinal distribution, and there are numerous cold water sea anemone species with pelagic offspring. Nonetheless, externally brooding species have not been described from tropical waters. Externally brooding southern hemisphere species (e.g., *Haliplanthella annularis* Carlgren 1938; *Cricophorus nutrix* (Stuckey 1909a); and *Epiactis georgiana*) occur only as far north as South Africa, and northern hemisphere species (e.g. *Urticina fecunda* and *Epiactis prolifera*) are primarily arctic and cold-temperate, but spread south to California (USA) and Japan (~37° N) in the North Pacific. That this form of brooding is primarily bipolar in distribution and that these species are representatives of multiple higher level taxonomic groups suggests there may yet be some ecological explanation (perhaps simply exclusion from the tropics) for the geographic distribution of externally brooding species, although the ACC hypothesis likely still plays a role in the Antarctic.

Evolution of brooding modes.

In the phylogeny we reconstruct (Figure 24), brooding overall has had multiple independent derivations, with internal brooding being more labile than external brooding. Within *Epiactis sensu stricto*, external brooding has evolved once from internally brooding ancestors. This seems intuitive, since internal brooding is in a sense a 'simpler' change from the free spawning norm (i.e., internal brooding requires only the delayed

release of offspring while external brooding requires the additional step, after release, of retaining offspring upon the surface of the adult by some mechanism). In this way we regard internal brooding as a conceptual, and perhaps actual, intermediate step between free spawning and external brooding. This view differs from that of Rodríguez et al. (2012b) who consider external brooding in the Antarctic species *E. georgiana* to be an intermediate state between the fully internal development of internal brooders on one hand and the fully external development of free spawners on the other. The interpretation suggested by Rodríguez et al. (2012b) is concordant with the phylogenetic reconstruction for the clade to which that species belongs (Figure 24), although their conception of “intermediate” is perhaps better interpreted as plesiomorphic: *E. georgiana* is sister to a clade that includes both internally brooding and spawning species.

There are few comparable studies in other marine invertebrate taxa that explicitly examine transitions between internal and external brooding modes in a phylogenetic context. Hart et al. (1997) found multiple independent origins of internal brooding in sea stars of the genus *Pateriella* Verrill 1913 but no externally brooding taxa (*sensu* this manuscript) were present in the analysis. A study by McFadden et al. (2001) of alcyonarian corals found that internal brooding was primitive to external brooding, but the presence of broadcast spawning species sister to the external brooders made the character transformation history ambiguous in the branch leading from internally brooding ancestors to the clade containing the externally brooding terminal taxon.

Our results concur with the historical assumption that brooding is a derived condition and that spawning is plesiomorphic (Chia 1974). Likewise, that brooding is never lost or

reversed in the lineage we examine supports the idea that a return to planktotrophy from lecithotrophic ancestors may be rare and difficult (Simpson 1953; but see Collin 2004). The evolution of external brooding from internally brooding ancestors in *Epiactis* is contrary with Chia's (1976) prediction that internal and external brooders would arise independently from oviparous-pelagic-lecithotrophs, though both groups do arise independently elsewhere in the tree. Our recovery of monophyly in *Epiactis* of the North Pacific contrasts with Edmands (1996), whose results always split *Epiactis* species with respect to *Anthopleura*.

Sex allocation.

As for brooding mode, the character states for sex allocation are generalizations. Hermaphrodites could be further subdivided into simultaneous, protandric sequential, protogynous sequential, gynodioecious, and possibly androdioecious variations. Some species are known to have male, female, and hermaphroditic individuals, with one or several modes being extremely infrequent (Rodríguez et al. 2012b). The great time and effort required to determine with certainty that a species consists of protandrous sequential hermaphrodites rather than dioecious individuals, for example, is complicated by the fact that size and age do not necessarily correlate in sea anemones. With a few exceptions of very thoroughly studied species, reports in the literature should be treated as 'at least' values. For these reasons, we consider species in which hermaphroditism is known and common (e.g. *Epiactis prolifera*) to be hermaphroditic and those in which it is unknown (e.g. *E. lisbethae*) or very infrequent (e.g. *E. georgiana*) to be gonochoric.

Edmands (1996) treated hermaphroditism specifically in terms of gynodioecy in *E. prolifera* and simultaneous hermaphroditism in *E. fernaldi* in her taxonomically more restricted analysis. There is insufficient data available to replicate that fine level of distinction across all or even most taxa in our analysis, but nevertheless we find sex allocation is extremely labile, even in general terms, and even within closely related species groups such as *Epiactis sensu stricto* (Figures 24, 28).

Hermaphroditism and brooding.

The correlation of brooding with hermaphroditism (and with small adult size) in marine invertebrates has long been recognized (Ghiselin 1969, Chia 1974, Strathmann and Strathmann 1982, Strathmann et al. 1984, Heath 1977, 1979). Any causal relationship between the two correlated features is uncertain however. Most proposed explanations assume that hermaphroditism is a response to brooding, or is enabled by it (Heath 1979, Strathmann et al. 1984). Heath (1979) explains hermaphroditism by the limited brooding space of females. If female reproductive output is limited by the physical space in which offspring can be accommodated, any excess energy that could otherwise be devoted to reproduction should be allocated to production of male gametes (Heath 1977). This hypothesis is consistent with the case of the gynodioecious life history of *Epiactis prolifera*, which broods externally. Since brood space is a factor of surface area (for external brooders) and gamete production is a factor of internal volume, egg production could outpace brood space as the animal grows. Correspondingly, individuals of this species begin life as female and later become hermaphroditic (Dunn 1975a). This is an

exceptional pattern however: the relatively larger species *E. lisbethae* and *E. ritteri* also brood externally but are gonochoric (Fautin and Chia 1986, Larson and Daly 2015). It should also be noted that the hypotheses of brood space limitation (or, alternatively, surplus reproductive energy) is in contradiction with Chia's "poor man's" hypothesis (1976) which supposes that reproductive energy is limited in brooding organisms rather than at a surplus. Strathmann et al. (1984) suggest that the low genetic diversity already present in brooding populations (due to reduced dispersal) lowers the relative genetic penalties incurred by hermaphroditism and self-fertilization, and hermaphroditism is therefore more likely to evolve in brooders. Inverting the causal relationship, if one exists, one might propose that hermaphroditism promotes the evolution of brooding through self-fertilization. While selfing provides an individual with fertilized eggs and may therefore lower some hypothetical barrier to brooding, it does not, in itself, explain why such an individual might then retain the zygotes through some developmental period. While brooding marine invertebrate species are disproportionately hermaphroditic, it is not clear that the reverse is true. Our results raise doubt about the strength of the correlation between brooding and hermaphroditism in sea anemones in particular.

Our results contrast with analyses in other hexacorallian groups. Kerr et al. (2011) reconstructed ancestral characters and examined character correlation between brooding and sex allocation in scleractinian corals. Like the present study, the ancestral state for the ingroup was nonbrooding gonochore with brooding and hermaphroditism being

derived conditions. Kerr et al. (2011), however, found significant dependence of transitions between brooding and spawning on those of reproductive mode.

It is important to consider the analytical implications of treating brooding as a categorical phenomenon (grouped by analogous behaviors) versus as an evolutionary phenomenon (with implications of homology across instances). The axiom that brooding in marine invertebrates is correlated with simultaneous hermaphroditism is of the former type, based on multiple independent observations in various taxa (Ghiselin 1969), but is generalized and conceals the complexity and diversity of the characters, especially those of brooding, in certain taxa. In the *Epiactis* system in particular, treating all forms (i.e., internal and external) of brooding as one state of a binary character (as is required by the correlation analysis) forces a categorical treatment upon a character in what is meant to be an evolutionary analysis. The result is that the origin of brooding is shifted to a single, ancestral node rather than the multiple later nodes from which the specific modes arise from in the multistate character reconstruction. Thus the number of times brooding is inferred to have evolved is reduced, and those transitions are moved among branches in the tree. Whether or not one detects a correlation between the evolution of sex allocation and brooding modes is dependent on how these characters are ancestrally reconstructed. With that undesirable effect in mind, we analyzed the correlation of hermaphroditism with each brooding mode separately and as a combined brooding character but found no significant evidence for a correlation in any case.

Our failure to detect such a general correlation may indicate either that it does not exist or is weak in sea anemones, or that the nature of this study biases taxon sampling towards

particularly evolutionarily labile lineages. A more thorough sampling of Actiniidae would be unlikely to alter the findings, however, because it would primarily disperse additional non-brooding species and genera among the taxa present in the analysis.

Conclusions

Due to the great diversity in morphology, sex allocation, and reproductive strategy coupled with its relative abundance and easily accessible intertidal habitat, the genus *Epiactis* promises to be fertile ground for the investigation of evolution, reproductive ecology, speciation, population structure, phenotypic diversity and many other relevant biological phenomena. For a start, *Epiactis sensu stricto* contains internal and external brooding, continual and seasonal reproduction, hermaphroditic, gonochoric, and gynodioecious sex allocation, selfing, outcrossing, sympatry, allopatry, various forms of brood protection and varying levels of phenotypic diversity with respect to color and color pattern. The establishment of relationships in a group with this much diversity is critical so that effects due to phylogeny can be avoided or exploited as needed by the questions under investigation.

While tree reconstruction methods differ slightly in their results, these differences affect none of the major findings of this project: 1. *Epiactis* is not monophyletic except for a group in the North Pacific which contains the type species and thus carries the generic name. 2. *Cnidopus* is not a valid genus and *C. ritteri* and *C. japonicus* should be referred to as *Epiactis ritteri* and *Epiactis japonica*, respectively. 3. External brooding is the derived condition from internally brooding ancestors in *Epiactis sensu stricto*.

Whether the geographical pattern for external brooding in sea anemones can be explained by adaptation for cold-temperate and polar climates, exclusion from tropical regions, or phylogeographic causes is currently unknown. Additional taxonomic sampling of polar and subpolar externally brooding species is needed to test such hypotheses. Present data, however indicates that external brooding has arisen in multiple independent lineages and persists only in these regions.

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