

博士論文

Phylogenetic and comparative morphological studies
of detritivorous calanoid copepods with highly
specialized sensory organs

特殊な感覚器官を持つデトリタス食性カラヌス目
カイアシ類の系統分類および比較形態学的研究

(要約)

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Introduction

Bradfordian families (Calanoida: Clausocalanoidea) are crustacean zooplankters that include seven families with 57 genera and are defined by a synapomorphy of specialized sensory setae on the maxilla, maxilliped, and sometime maxillule (Ferrari & Steinberg, 1993). Bradfordian taxa appear in all depth layers of the marine environment, and most of them have been considered detritivores. Bradfordian families have specialized sensory organs that seem to be associated with the detection of detrital foods. The specialized chemosensory setae are categorized into two types: brush-like setae with apertures on their tips and worm-like setae without apertures. Nishida and Ohtsuka (1997) and Chan (2021) investigated the ultrastructure of these sensory setae in two scolecitrichid and three phaennid species. They detected one dendrite with hundreds of cilia on it and some enveloping cells in the proximal part of each sensory seta. In addition, some Bradfordian taxa also have specialized optical sensory organs. Nishida et al. (2002) studied the large optical sensory organs of the phaennid *Cephalophanes* and found that these organs have a pair of semi-parabolic reflectors that can gather light in a wide range of wavelengths. They suggested that these organs detect the bioluminescence of the bacteria attached to crustacean detrital foods. Although the gross morphology of these sensory organs is diversified (Bradford, 1973), the functions of sensory organs have been presumed in only a limited number of representative taxa.

The diversification of the Bradfordian niches during evolution is very attractive; however, the confusion of taxonomy and phylogeny of the group is a serious challenge. The definitions of some families remain unclear (Markhaseva et al., 2014), and molecular phylogeny has not revealed relationships among the Bradfordian genera (Laakmann. et al., 2019). Therefore, the conventional classification of the families is questionable. Thus, a phylogenetic revision of the group is necessary for studying the evolution of Bradfordian taxa.

In the present study, sensory functions of multiple Bradfordian taxa were presumed from the observation of the ultrastructure of the chemosensory setae and optical sensory organs. In addition, the phylogeny of the Bradfordian taxa and their colonization routes are discussed. Furthermore, Bradfordian feeding niches were compared among taxa based on bacterial floras of gut contents.

Ultrastructure and possible functions of chemosensory setae on the maxillae

Bradfordian taxa were collected from epi- and mesopelagic layers of the Kuroshio region by using ORI and sledge nets in 2016–2019. Bradfordian chemosensory setae were observed using scanning (SEM) and transmission (TEM) electron microscopes following the method described by Ohtsuka et al. (2021).

In addition to sensory cilia reported by previous studies (Nishida and Ohtsuka, 1997; Chan, 2021), cells similar to solitary chemosensory cells (SCCs) and free nerve endings (FNEs) were newly discovered in the chemosensory setae of scolecitrichids (Fig. 1). The SCC-like cells and

FNEs have not been observed in any arthropod aesthetascs and are similar to vertebrate chemosensory cells. In contrast to sensory cilia, SCCs and FNEs in vertebrates can sustain high sensory abilities for a long time (Kashiwayanagi & Kurihara, 1994). Those of the Bradfordian taxa may continuously search for odorous food items. In contrast to scolecitrichids, phaennids developed sensory cilia and FNEs instead of SCC-like cells (Chan, 2021). The ultrastructure of the worm-like setae of scolecitrichids without the optical sensory organs (*Amallothrix valida* and *Pseudoamallothrix emarginata*) was different from that of scolecitrichids with well-developed optical sensory organs (*Macandrewella stygiana*, *Scolecithrix danae*, *Scottocalanus securifrons* and *Lophothrix frontalis*). The cavity of the worm-like setae of *A. valida* and *P. emarginata* was large and contained many SCC-like cells (Fig. 1C). These two species may have developed worm-like setae because of the absence of light sensation.

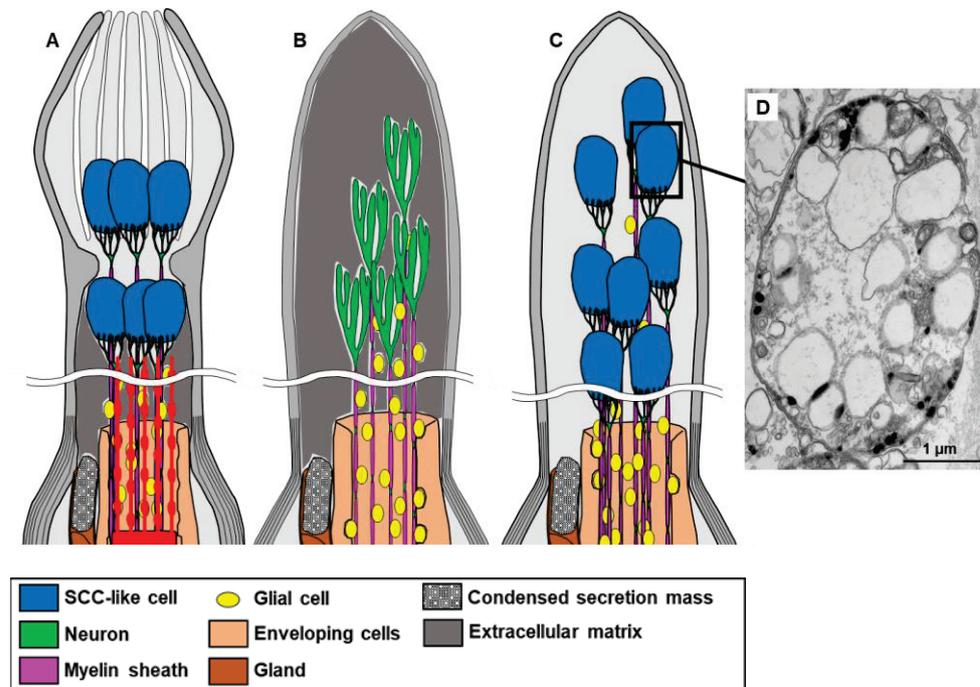


Fig. 1. Schematic illustrations of the chemosensory setae of scolecitrichids. (A) Brush-like setae. (B) Worm-like setae of *Scottocalanus securifrons* and *Lophothrix frontalis*. (C) Worm-like setae of *Amallothrix valida* and *Pseudoamallothrix emarginata*. (D) TEM micrograph of SCC-like cells (longitudinal section) in the worm-like setae of *Amallothrix valida*.

Ultrastructure and possible functions of optical sensory organs

Scottocalanus securifrons had a single ventral and paired dorsal ocelli in the naupliar eyes as well as paired Gicklhorn's organs, whereas *M. stygiana* lacked Gicklhorn's organs, and *L. frontalis* and *Phaenna spinifera* lacked dorsal ocelli. The dorsal ocelli of *S. securifrons* and all ocelli of *M. stygiana* faced cuticular lenses. The Gicklhorn's organ located on the dorsal, lateral and/or anterior sides developed in pelagic taxa (*S. securifrons*, *L. frontalis*, and *P. spinifera*), enabling them to search for detrital foods in a three-dimensional environment.

Although copepod ocelli generally have dense rhabdoms that are located on the inner most part (Umminger, 1968; Elofsson, 1970), most Bradfordian ocelli, except for the naupliar eyes of *S. securifrons* and *M. stygiana*, were filled with large rhabdoms. Enlarged rhabdoms in these ocelli may sharpen their sensory abilities.

There were phaosomes and densely packed rhabdoms in the naupliar eyes of *S. securifrons* and *M. stygiana*, and these organelles were located symmetrically in the naupliar eyes. Optical path analysis based on Snell's law indicated that these cuticular lenses gather light to the phaosomes but not to the rhabdoms. Results of optical path analysis of the phaosomes imply that they play a role in optical waveguides (Fig. 2).

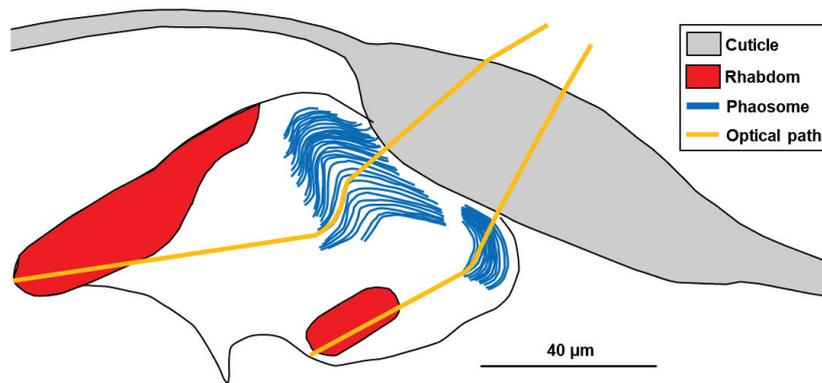


Fig. 2. Possible optical paths in the ventral ocellus of *Macandrewella stygiana*.

Taxonomy, phylogeny and colonization routes of Bradfordian families

Previous descriptions of various Bradfordian taxa (40 genera with 40 species) were used here for the phylogenetic analysis on the basis of the morphological data. The hypothetical ancestor was established based on the newly described diaixid *Pogonura rugosa* (Komeda & Ohtsuka, 2020) and some other primitive Bradfordian genera. Seventy-eight irreversible and 10 unordered characters were used for this analysis. The phylogenetic analysis was established with PAUP 4.0a169.

The monophyly of the phaennid clade (Cl-7 in Fig. 3) was strongly supported by a high jackknife value (93%). In addition, the scolecitrichid genera, except *Diaiscolecithrix* and *Omorius*, were also grouped with a high jackknife value (80%, Cl-6 in Fig. 3).

The Bradfordian colonization route is also discussed (Fig. 4). A hypothetical ancestor of the Bradfordian taxa is perceived as a deep-sea hyperbenthic copepod, and the colonization of the pelagic realm seems to have arisen independently at least four times. Furthermore, some scolecitrichid genera are presumed to recolonize the hyperbenthic layer from the pelagic realm. The Bradfordian colonization routes may become more complicated than those of arietellids (non-Bradfordian calanoids) that have been well investigated (Komeda et al., 2021).

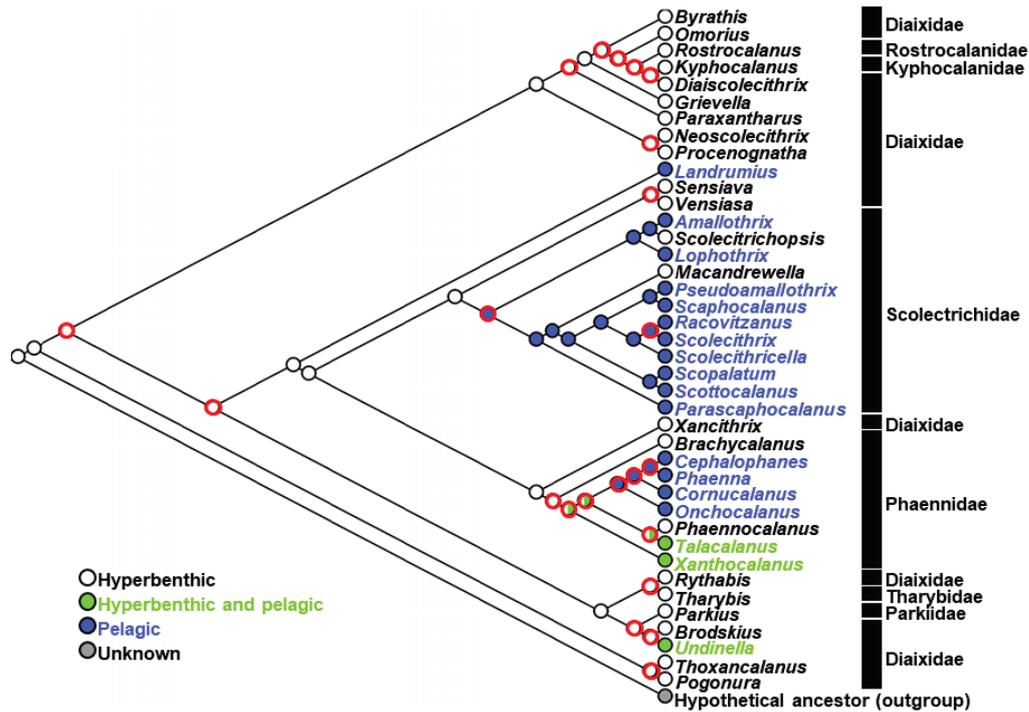


Fig. 3. Most parsimonious tree (50% majority-rule consensus) with colonization routes of Bradfordians inferred using “Trace Character History” in Mesquite ver. 3.70.

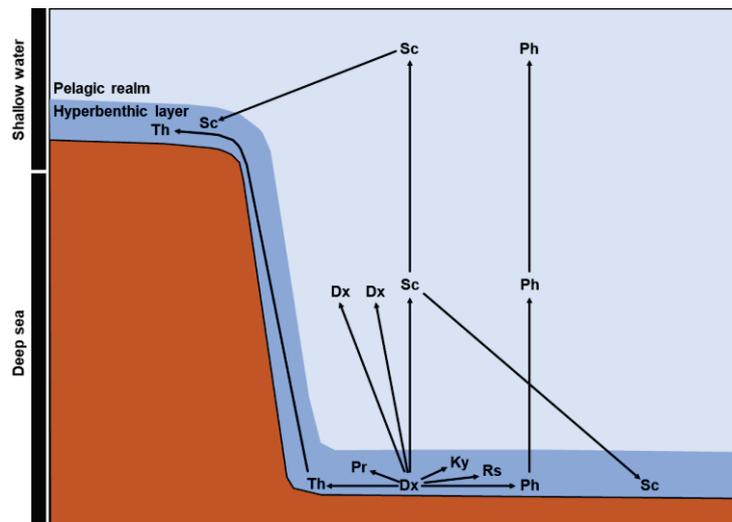


Fig. 4. Hypotheses of the Bradfordian colonization route. Dx: Diaixidae, Ky: Kyphocalanidae, Ph: Phaennidae., Pr: Parkiidae, Rs: Rostrocalanidae, Sc: Scolecitrichidae, Th: Tharybidae.

Feeding habits based on enteric bacterial flora

To analyze the enteric bacterial flora, gut contents of six scolecitrichid species were used for 16S rRNA metabarcoding. The bacterial flora was significantly different among the scolecitrichids with and without optical sensory organs (analysis of similarities based on the Chao index, $p < 0.01$). Principal component analysis suggested that most scolecitrichid specimens with the optical sensory organs correlated with bioluminescent Vibrionaceae (*Photobacterium* and

Vibrio), Planctomycetes, and *Synechococcus* (Fig. 5). These scolecitrichids may specialize in sensing detrital food with bioluminescent bacteria such as the Vibrionaceae. In contrast, the enteric bacterial flora of the scolecitrichids without the optical sensory organs differed among specimens, and some specimens strongly correlated with non-bioluminescent genera such as *Pseudoalteromonas*, *Sinobacterium*, *Umboniibacter*, and/or *Tenacibaculum* (Fig. 5). These scolecitrichids may depend on various odorous detrital matters with acyl-homoserine lactone (AHL)-producing bacteria such as *Pseudoalteromonas* and *Tenacibaculum*, because they had several SCC-like cells in their worm-like setae and AHLs are known as key chemical stimuli detected by vertebrate SCCs (Zheng et al., 2019).

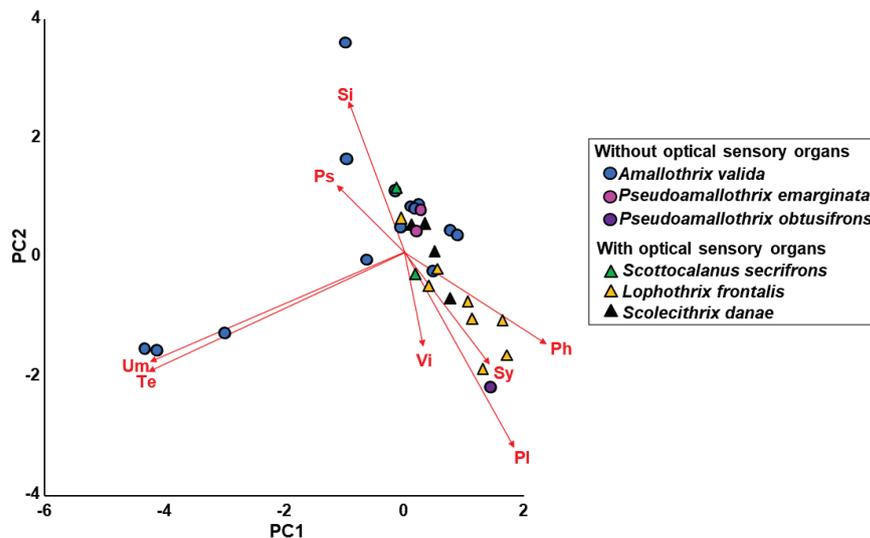


Fig. 5. Principal component analysis of bacterial flora found in the gut contents of six scolecitrichid species. Principal component loadings of each bacterial taxon are shown as red arrows. Ph: *Photobacterium*, Pl: Planctomycetes, Ps: *Pseudoalteromonas*, Si: *Sinobacterium*, Sy: *Synechococcus*, Te: *Tenacibaculum*, Um: *Umboniibacter*, Vi: *Vibrio*.

General discussion

Bradfordian specialized multiple types of sensory organs may allow them to search for a variety of detrital foods in oligotrophic environments. These organs have complementarily developed, and the taxa with optical sensory organs seem to mainly search for food items with bioluminescent bacteria, whereas others may detect odorous bacterial signals originating from foods. Furthermore, these organs seem to be specialized to different habitats (Fig. 6). In conclusion, Bradfordian sensory organs seem to have been diversified through an adaption to different habitats and feeding niches, and this niche partitioning has maintained in the Bradfordian diversity in oligotrophic waters.

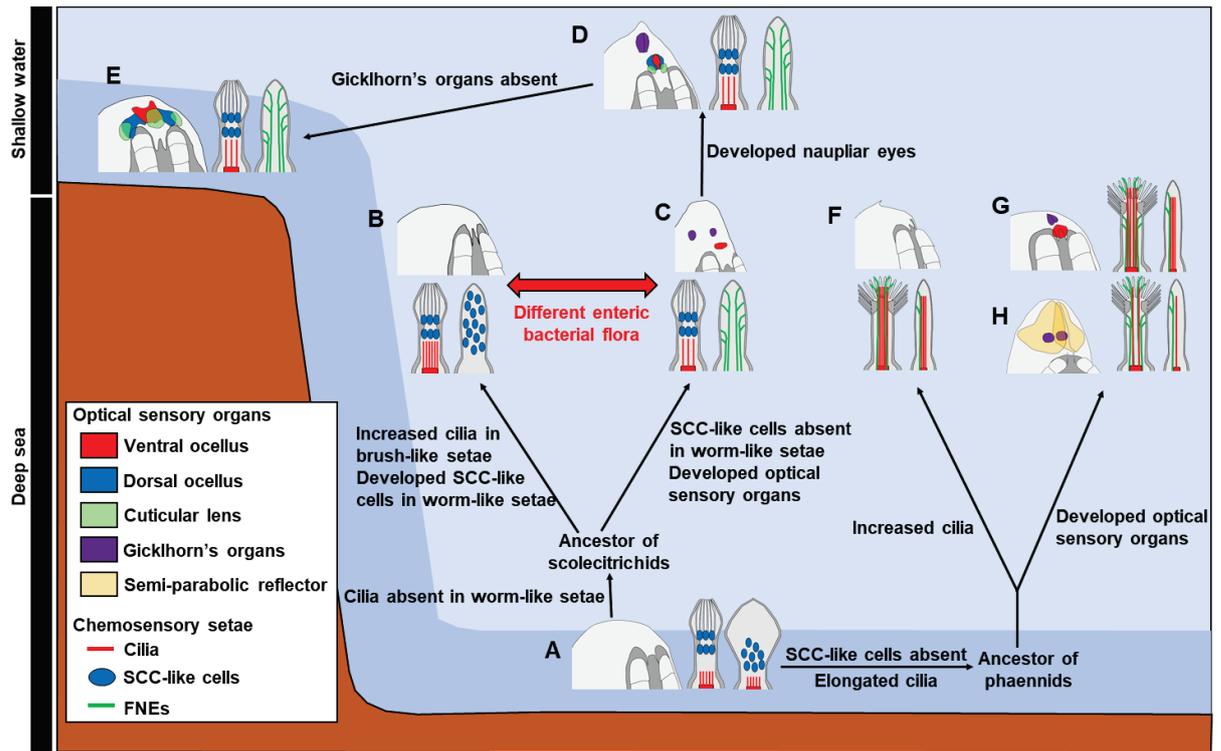


Fig. 6. Possible adaptive radiation of Bradfordian taxa based on ultrastructure of sensory organs.

(A) Deep-sea hyperbenthic diaixids (*Neoscolecithrix japonica*). (B, C) Mesopelagic scolecitrichids without (*Amallothrix valida* and *Pseudoamallothrix emarginata*) and with (*Lophothrix frontalis*) optical sensory organs, respectively. (D) Epipelagic scolecitrichids (*Scolecithrix danae* and *Scottocalanus securifrons*). (E) Shallow-water hyperbenthic scolecitrichids (*Macandrewella stygiana*). (F–H) Mesopelagic phaennids investigated by Nishida et al. (2002) and Chan (2021) (*Cornucalanus chelifera*, *Phaenna spinifera*, and *Cephalophanes refulgens*, respectively).