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# Comparative morphology among trunk limbs of *Caenestheriella gifuensis* and *Leptestheria kawachiensis* (Crustacea: Branchiopoda: Spinicaudata)

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Morphological differences among groups of the 24 trunk limbs of *Caenestheriella gifuensis* (Ishikawa, 1895) and differences between males and females are described and illustrated. A setose attenuate lobe located proximally near enditic lobe 1 and a discoid lobe covered with small setae proximal to enditic lobe 1 are newly described. The five ventral enditic lobes, endopod, exopod, and dorsal exite of traditional spinicaudatan morphology are redescribed. Trunk limbs 1–4 of females bear a palp on enditic lobe 5 and trunk limbs 1–15 of males bear a similar palp. A second, articulating palp is associated with the base of the endopod of trunk limbs 1–2 of males. The proximal part of trunk limbs 19–24, bearing enditic lobe 1, articulates by an arthrodial membrane with the remainder of the limb, and the exite is distal to this arthrodial membrane. Development of trunk limbs, ascertained through an examination of early juvenile instars of *Leptestheria kawachiensis* Uéno, 1927, includes an asetose limb followed in time by a series of setose limbs that increase in morphological complexity with age. The number of lobes on the asetose limb varies from seven (corresponding to five enditic lobes, an endopod, and an exopod) on anterior limbs to five on trunk limb 24, which lacks the lobes corresponding to enditic lobe 4 and the endopod; these two structures are added later to setose limbs. The attenuate lobe, the discoid lobe, the exite of all trunk limbs, and the palps of the anterior trunk limbs are added to the setose limbs. Development of anterior limbs is accelerated relative to that of posterior limbs, and development of the more posterior limbs is truncated relative to that of limbs immediately anterior to them. Enditic lobe 4 and the endopod of limbs like trunk limb 24 develop from, or are patterned by, enditic lobe 5; the articulating palp of male trunk limbs 1–2 also may be added in this way. A comparison of these observations with development of the copepod maxilliped suggests that the spinicaudatan trunk limb is composed of a praecoxa with three lobes, a coxa and a basis each with one lobe, and an endopod of three segments in females and four in males. This is similar to the homology scheme previously proposed by Hansen in 1925. A critique is given of attempts to homologize parts of arthropod limbs based on developmental gene expression patterns. Stenopodal to phyllopodal transformations of maxillipeds in copepods provide a model at least partly applicable to spinicaudatans, and a 'multibranched' interpretation of spinicaudatan (and by extension branchiopodan) limb morphology is rejected. There is nothing intrinsic to the structure of the adult trunk limbs suggesting that they are similar to the adult limbs of the ancestral branchiopod or the ancestral crustacean, but early developmental steps of more posterior limbs are good matches for the morphology of an ancestral crustacean biramal limb predicted by a hypothesis of duplication of the proximo-distal axis.

ADDITIONAL KEYWORDS: appendages development – homologies – spinicaudatan.

## INTRODUCTION

Trunk limbs of branchiopods have been described as: phyllopodal, and uniform or similar in shape (McLaughlin, 1980: 8; Calman, 1909: 29); posteriorly decreasing in size (Brusca & Brusca, 1990: 607), or size and complexity (Fryer, 1987: 374), or size and development (Schram, 1986: 380); multibranched (Nulsen & Negy, 1999; Williams, 1999); or, in the case of spinicaudatans, serially similar but differing in size (Martin, 1992: 35). In this paper we describe and compare the morphological differences among

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*Corresponding author. E-mail: ferrari.frank@msc.si.edu trunk limbs of adult *Caenestheriella gifuensis* (Ishikawa) [Cyzicidae] and differences between its males and females. The initial few steps in the development of several trunk limbs of *Leptestheria kawachiensis* Uéno from a second family, Leptestheriidae, are described to determine the extent to which the above descriptions apply to the trunk limbs of these two spinicaudatans. We consider whether posterior trunk limbs of adults of *C. gifuensis* are miniature anterior trunk limbs. We also compare the development of anterior and posterior trunk limbs of *L. kawachiensis* to determine whether smaller and ontogenetically younger posterior trunk limbs are anterior trunk limbs whose development has been truncated by the lack of developmental time, i.e. whether they would take on the morphology of larger anterior trunk limbs if they had more time to develop. We discuss homologies of limb segments suggested by the developmental patterns of these trunk limbs, whether these kinds of limbs should be considered similar to those of the ancestral crustacean, and the appropriateness of describing the spinicaudatan (and, by extension, the branchiopodan) trunk limb as multibranched.

## MATERIAL AND METHODS

Large adult specimens of *Caenestheriella gifuensis* were collected on 11 June 2001 from a rice paddy in Suikei-cho, Omi-Hachiman-shi, Shiga-ken, Japan; small adult specimens of *C. gifuensis* were collected on 24 May 2001 from a rice paddy in Kamitanakami-nakano-cho, Otsu-shi, Shiga-ken. Developmental stages including adults of *Leptestheria kawachiensis* were collected from rain puddles in harvested rice paddies in Kataoka-cho, Kusatsu-shi, Shiga-ken, by repeated sampling for 3 weeks beginning on 16 September 1999, or recovered from a plastic tray in which ovigerous females, collected from rice paddies in Kataoka-cho or Oroshimo-cho, Kusatsu-shi, had been kept between 15 May and 25 September 1999. After the death of the last adult in October 1999, the water in the tray was allowed to evaporate. The tray was refilled with filtered water from Lake Biwa in October 2000, and developmental stages and adults were collected from 25 October 2000 to 8 December 2000.

Specimens selected for study (Table 1) were identified by length and height of the carapace. The first four groups of specimens of *L. kawachiensis*, lacking a car-

**Table 1.** Specimens examined, listed by the number of setose limbs present. A, greatest length of carapace; B, greatest height of carapace; C, number of carapace rings; D, carapace relative to body; E, number of setose limbs; F, number of asetose limbs; G, number of limb stripes; H, sex [female, with eggs; male, with transformed trunk limbs 1–2]

| A | B | C | D | E | F | G | H |
|---|---|---|---|---|---|---|---|
| *Caenestheriella gifuensis* | | | | | | | |
| 7.6 | 4.9 | 18 | enclosing body | 24 | 0 | 0 | male |
| 8.7 | 5.4 | 20 | enclosing body | 24 | 0 | 0 | female |
| 5.3 | 3.3 | 11 | enclosing body | 24 | 0 | 0 | male |
| 5.4 | 3.4 | 11 | enclosing body | 24 | 0 | 0 | female |
| *Leptestheria kawachiensis* | | | | | | | |
| 0 | 0 | 0 | absent | 0 | 0 | 3 | |
| 0.1 | 0.3 | 0 | not enclosing | 0 | 0 | 8 | |
| 0.2 | 0.2 | 0 | not enclosing | 5 | 2 | 2 | |
| 0.3 | 0.3 | 0 | not enclosing | 5 | 2 | 2 | |
| 0.7 | 0.6 | 0 | enclosing body | 9 | 2 | 2 | |
| 0.8 | 0.6 | 0 | enclosing body | 10 | 2 | 1 | |
| 1.0 | 0.8 | 1 | enclosing body | 11 | 2 | ? | |
| 1.5 | 0.8 | 2 | enclosing body | 13 | 3 | ? | |
| 1.5 | 1.0 | 4 | enclosing body | 15 | 2 | 1 | |
| 2.0 | 1.1 | 5 | enclosing body | 15 | 2 | 2 | |
| 1.9 | 1.3 | 5 | enclosing body | 17 | 2 | 2 | |
| 2.5 | 1.6 | 10 | enclosing body | 20 | 1 | 2 | |
| 2.3 | 1.6 | 9 | enclosing body | 21 | 3 | 0 | |
| 2.8 | 1.7 | 9 | enclosing body | 22 | 2 | 0 | |
| 3.9 | 2.3 | 12 | enclosing body | 24 | 0 | 0 | female |
| 4.6 | 2.9 | 13 | enclosing body | 24 | 0 | 0 | male |
| 8.0 | 4.7 | 15 | enclosing body | 24 | 0 | 0 | female |
| 8.3 | 4.3 | 13 | enclosing body | 24 | 0 | 0 | male |

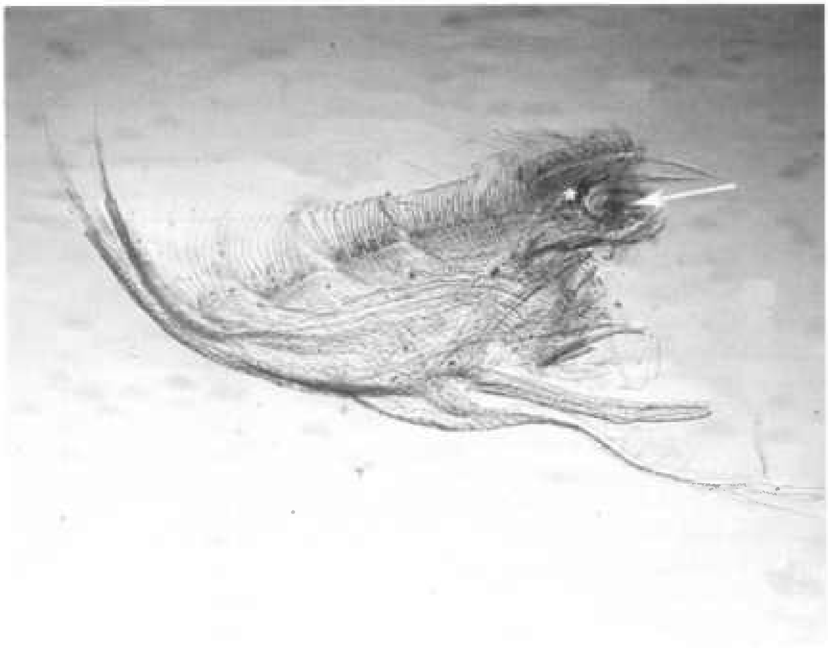

**Figure 1.** *Caenestheriella gifuensis* adult female (8.7 mm × 5.4 mm). Trunk limb 13, anterior. Ventral up, proximal right; arrow to discoid lobe; asterisk on base of attenuate lobe.

apace or with a carapace that does not enclose the body, were considered nauplii because each has a naupliar arthrite on the protopod of antenna 2. We considered as three successive developmental stages the specimens of *L. kawachiensis* with: nine, ten or 11 setose limbs; 13 or 15 setose limbs, and 21 or 22 setose limbs, respectively, although there are slight differences in setation among the specimens within each group, and the possibility exists that each represents more than one stage. In the earliest juveniles there are as yet no growth rings on the carapace to help in staging them exactly. Two size morphs of females with eggs or males with transformed trunk limbs 1–2 were found for both species. Differences in these morphs are described briefly with the adult morphology.

Specimens initially were cleared in glycerin for measurements of the carapace. The valves of the carapace then were dissected, and the valves and body transferred to lactic acid and stained by adding a solution of chlorazol black E dissolved in 70% ethanol/30% freshwater. Chlorazol black E adheres to the exoskeleton of crustaceans (Cannon, 1937, 1941), including the growth rings of the carapace; it also adheres to the nuclear membrane of cells. Because the exoskeleton of the trunk limbs of these branchiopods is so poorly sclerotized, staining times at room temperature often exceeded an hour; in most cases staining was accelerated by heating with a beverage warmer. Trunk limbs were examined with bright-field and with differential interference optics. Trunk limbs of spinicaudatans bear many, closely spaced setae (Fig. 1); no attempt was made to provide exact counts of these setae. In Figures 2–8 most setae are not drawn so that limb morphology can be observed more easily. Drawings were made with a camera lucida with the exception of Figure 1, which is a montage composite prepared by analysing four successive digital images of a trunk limb captured at different Z-axis focal plane levels.

## TERMS

Trunk limbs of spinicaudatans and related branchiopods usually are proximodistally elongate and anterioposteriorly flattened. Structures originating on the surfaces of the third axis of the limb often are described as lateral (or outer) and medial (or inner); the descriptors dorsal and ventral are used here, respectively, following Cohen (1993). Trunk limbs of spinicaudatans often are described as having five ventral enditic lobes, a terminal endopod, a dorsal exopod, and a dorsal epipod (Schram, 1986; Gruner, 1994; Olesen, Martin & Roessler, 1996), although other systems have been proposed (Hansen, 1925; Shakoori, 1968; Walossek, 1993). These terms are used in the descriptions here except that the more general term 'exite' for an articulating dorsal projection of the protopod is substituted for 'epipod', which is an articulating dorsal projection of the coxa. A palp in the present context is a poorly sclerotized extension of the exoskeleton, usually with a crown of small setae, originating within the ellipse of setae of an enditic lobe toward the distal edge of its ventral face. Setae are socketed, articulating elements of an appendage and their morphology among spinicaudatan trunk limbs is diverse; here setal morphology is not described. Setae that are orientated in the same direction are described as polarized. Denticles, which are epicuticular extensions of an appendage segment, are neither described nor figured.

The first step in limb development recognized in this study is a dark, transverse stripe on the trunk, consisting of densely packed cells with stained nuclei, the presumptive trunk limb. Other steps include one asetose limb, in which the primary axis of the limb (presumably proximodistal) is deflected dorsally along the somite, and several setose-limb steps in which the primary axis of the limb, proximodistal, appears ventral to the body. A setose limb has at least one seta. Two steps, the asetose-limb step and the initial setose-limb step, are described in the development of trunk limb 6 of *L. kawachiensis*; four steps are described for trunk limb 13 of this species, i.e. until all enditic lobes, rami, and the exite are present and the setation has become sufficiently complex that further steps could not be usefully compared. For the more posterior limbs, five steps for trunk limb 19 and three steps for trunk limb 24 are described, from the largest specimen obtained.

## RESULTS

Carapace measurements, number of carapace rings, and number of kinds of trunk limbs of adults (females with eggs or males with transformed trunk limbs 1–2) of *C. gifuensis*, and adults, juveniles, and nauplii of *L. kawachiensis*, are given in Table 1. Trunk limb 13 of an adult female *C. gifuensis* (8.7 mm × 5.4 mm) is representative of an anterior trunk limb (Fig. 2A). It is poorly sclerotized with the exception of the setae, and there are no arthrodial membranes. Inside a trunk limb, setae often can be observed on the post-moult limb, the limb that will appear after the next moult. Enditic lobe 1 is bent proximally. There is an attenuate lobe proximally near enditic lobe 1, and a discoid lobe covered with small setae proximal to enditic lobe 1. The setae on enditic lobes 2–5 are polarized ventrally, and arranged in a proximally open ellipse with the major axis orientated proximodistally along the face of the enditic lobe (Fig. 2D). The line of posterior setae continues along the proximal edge of each enditic lobe; the posterior setae are thicker than the anterior setae. The rami have setae along both the dorsal and the ventral margins. The exopod has a proximal and a distal lobe; the proximal lobe is slightly shorter than the exite.

More anterior trunk limbs are progressively larger than trunk limb 13 (Table 2). Trunk limbs 1–5 are about the same size, but the exoskeleton forms a narrow constriction between enditic lobe 5 and the endopod (Fig. 2B). Trunk limb 1 has a posterior knob covered with denticles at the base of the exopod and distal to the exite. Trunk limbs 1–4 have a palp originating toward the distal edge of the ventral face of enditic lobe 5 within the ellipse of setae. The palp of trunk limbs 1–3 is similar in size (Fig. 2B); that of trunk limb 4 is very small (Table 2) and bears two setae (see Fig. 2C).

**Table 2.** *Caenestheriella gifuensis*. Length (L) of trunk limb from proximal edge of enditic lobe 1 to tip of endopod, and length of palp (P) on enditic lobe 5, of all 24 trunk limbs of gravid female of carapace dimensions 8.7 mm × 5.4 mm, and of 13 non-transformed, palp-bearing trunk limbs (3–15) of male of carapace dimensions 7.6 mm × 4.9 mm; t, transformed trunk limb; s, only two setae

| | Female | | Male | |
|---|---|---|---|---|
| | L | P | L | P |
| 1 | 2.97 | 0.61 | t | t |
| 2 | 3.13 | 0.67 | t | t |
| 3 | 3.17 | 0.67 | 2.51 | 0.94 |
| 4 | 3.15 | 0.04 | 2.71 | 1.17 |
| 5 | 3.18 | | 2.71 | 1.17 |
| 6 | 3.02 | | 2.64 | 1.10 |
| 7 | 2.90 | | 2.64 | 0.91 |
| 8 | 2.71 | | 2.33 | 0.58 |
| 9 | 2.63 | | 2.32 | 0.30 |
| 10 | 2.48 | | 2.10 | 0.13 |
| 11 | 2.36 | | 2.04 | 0.06 |
| 12 | 2.18 | | 1.81 | 0.05 |
| 13 | 2.13 | | 1.65 | 0.02 |
| 14 | 1.57 | | 1.42 | 0.01 |
| 15 | 1.36 | | 1.20 | s |
| 16 | 1.21 | | | |
| 17 | 1.04 | | | |
| 18 | 0.91 | | | |
| 19 | 0.75 | | | |
| 20 | 0.52 | | | |
| 21 | 0.49 | | | |
| 22 | 0.39 | | | |
| 23 | 0.29 | | | |
| 24 | 0.22 | | | |

Trunk limbs posterior to 13 also exhibit morphological differences. Enditic lobe 1 of trunk limbs posterior to 14 is well separated from the remaining lobes. Enditic lobe 1 of trunk limbs posterior to 16 is not bent proximally. The part of the limb bearing enditic lobe 1 of trunk limbs posterior to 18 articulates by an arthrodial membrane with the remainder of the limb, and the attenuate lobe is represented only by setae (Fig. 3A). The exite is distal to the arthrodial membrane. The endopod of trunk limbs posterior to 21 is

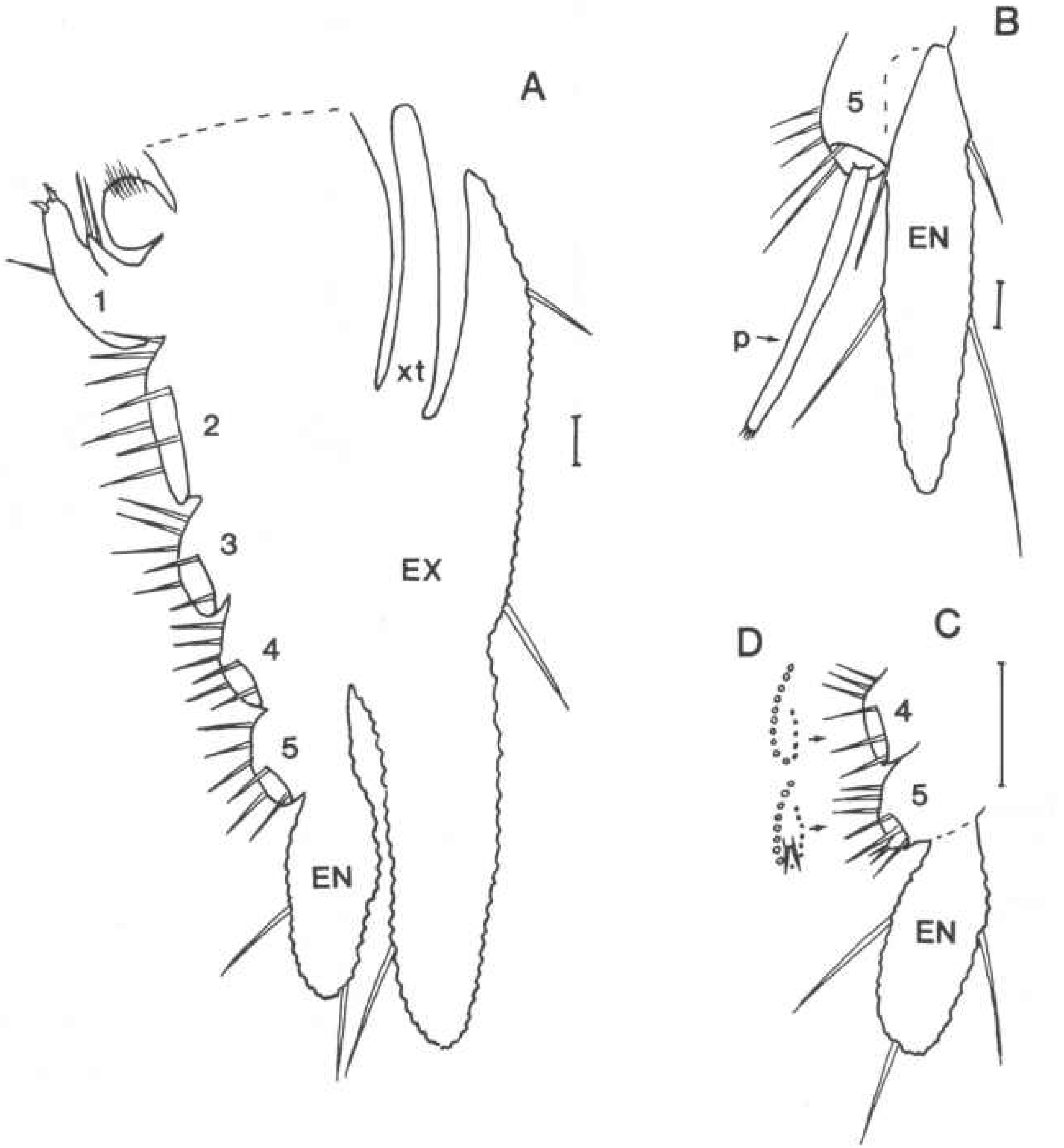

**Figure 2.** *Caenestheriella gifuensis* adult female (8.7 mm × 5.4 mm). A, trunk limb 13, anterior. B, trunk limb 2, enditic lobe 5 and endopod, anterior. Adult male (7.6 mm × 4.9 mm). C, trunk limb 15, endopod and enditic lobes 4–5, anterior, with small palp bearing 2 setae. D, ventral face of enditic lobes 4–5 of trunk limb 15 showing position of palp, anterior setae (closed circles), and posterior setae (open circles). Scale bars: 0.1 mm. For A–C, ventral left, proximal up; in A, broken line indicates area of attachment to the body; enditic lobes 1–5 are numbered; EN, endopod; EX, exopod; P, palp; xt, exite.

not distinctly separate from enditic lobe 5, and the discoid lobe is represented by a knob (Fig. 3B). The exopod of trunk limb 24 has only dorsal and terminal setae; the exite is a knob and there is no endopod, and the setae of enditic lobes 2–3 are not polarized ventrally. A summary of differences among groups of trunk limbs is found in Table 3.

Trunk limb 13 of an adult male (7.6 mm × 4.9 mm) is similar to that of the female but there is a palp toward the distal edge of the ventral face of enditic

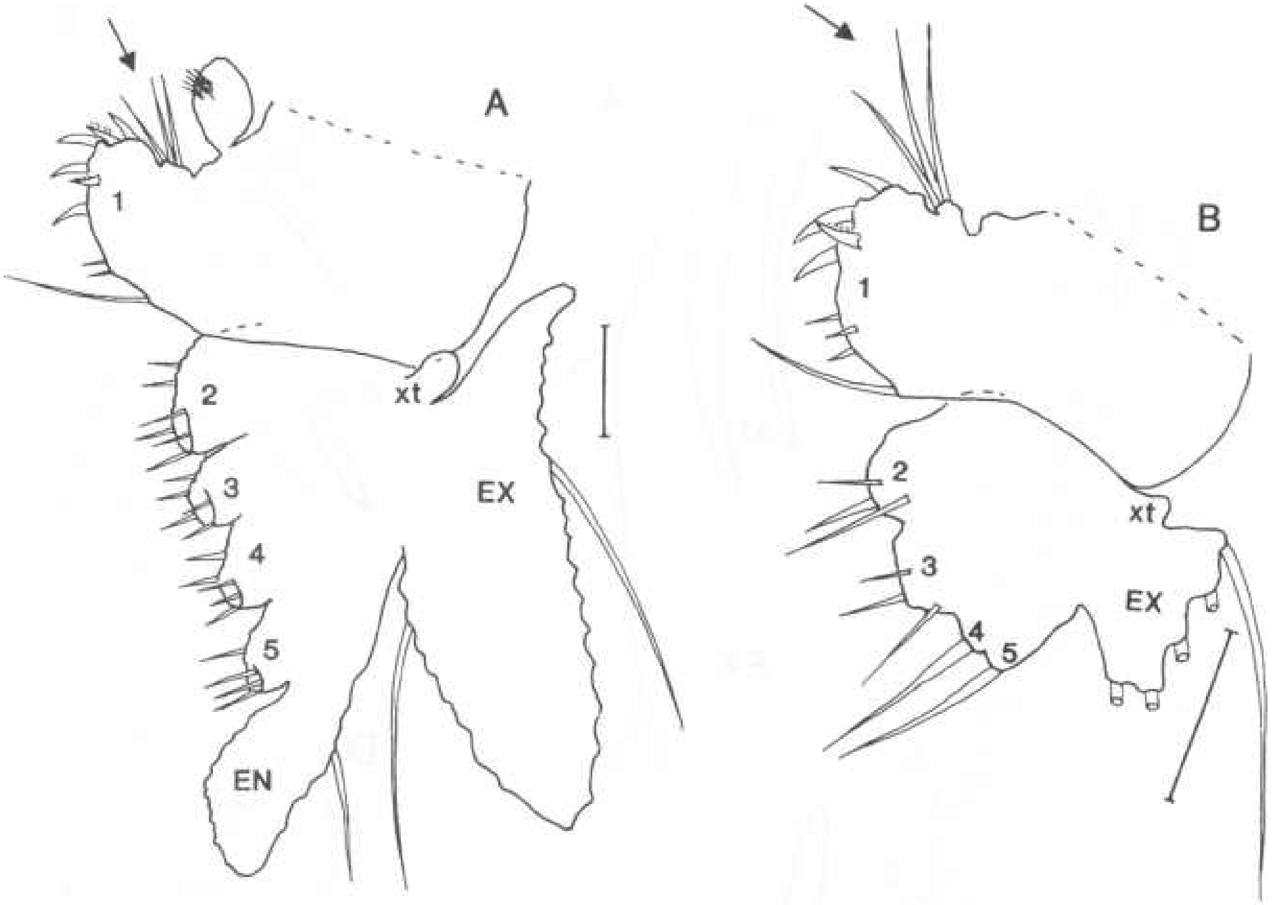

**Figure 3.** *Caenestheriella gifuensis* adult female (8.7 mm × 5.4 mm). A, trunk limb 19, anterior. B, trunk limb 24, anterior. Arrow on setae of presumptive attenuate lobe. Abbreviations and symbols as for Fig. 2.

**Table 3.** Occurrence of selected structures on trunk limbs 1–24 of adult female *Caenestheriella gifuensis*

| Structure | Trunk limbs |
|---|---|
| Posterior knob at base of exopod | 1 |
| Palp on enditic lobe 5 | 1–4 |
| Constriction at base of endopod | 1–5 |
| Enditic lobe 1 bent proximally | 1–16 |
| Attenuate lobe developed | 1–18 |
| Discoid lobe developed | 1–21 |
| Dorsal setae on endopod | 1–21 |
| Endopod present | 1–23 |
| Endopod well separated from enditic lobe 5 | 1–21 |
| Ventral setae on exopod | 1–23 |
| Arthrodial membrane distal to enditic lobe 1 | 19–24 |

lobe 5. A similar palp also is found on more anterior trunk limbs; on trunk limbs 14–15 it is represented by two setae and lacks the poorly sclerotized extension of the exoskeleton (Fig. 2C). The general trend of limb and palp diminution in size from anterior to posterior is shown in Table 2 for non-transformed, palp-bearing trunk limbs 3–15. Adult male trunk limbs 1–2 are similar to each other, and each possesses an exopod and an exite dorsally (Fig. 4A). The ventral margin of each limb can be divided into three parts. Enditic lobe 1 bends proximally, the attenuate lobe is relatively larger than that of the female, and a discoid lobe is present. This part of the limb is well sclerotized. The middle part is poorly sclerotized and consists of three setae-bearing lobes that may be distorted by muscle contraction; the exite and exopod are attached dorsally on this part of the limb. Trunk limb 1 (not illustrated) has a posterior knob covered with denticles at the base of the exopod and distal to the exite, as on the limb of the female; trunk limb 2 does not. The distal part of the limb is well sclerotized and consists of two articulating sections (Fig. 4B), each with an associated palp. The proximal section is broad with a palp originating ventrally and distally, near a group of short setae. The distal section is thinner and curved, its tip meeting the group of short setae of the proximal sec-

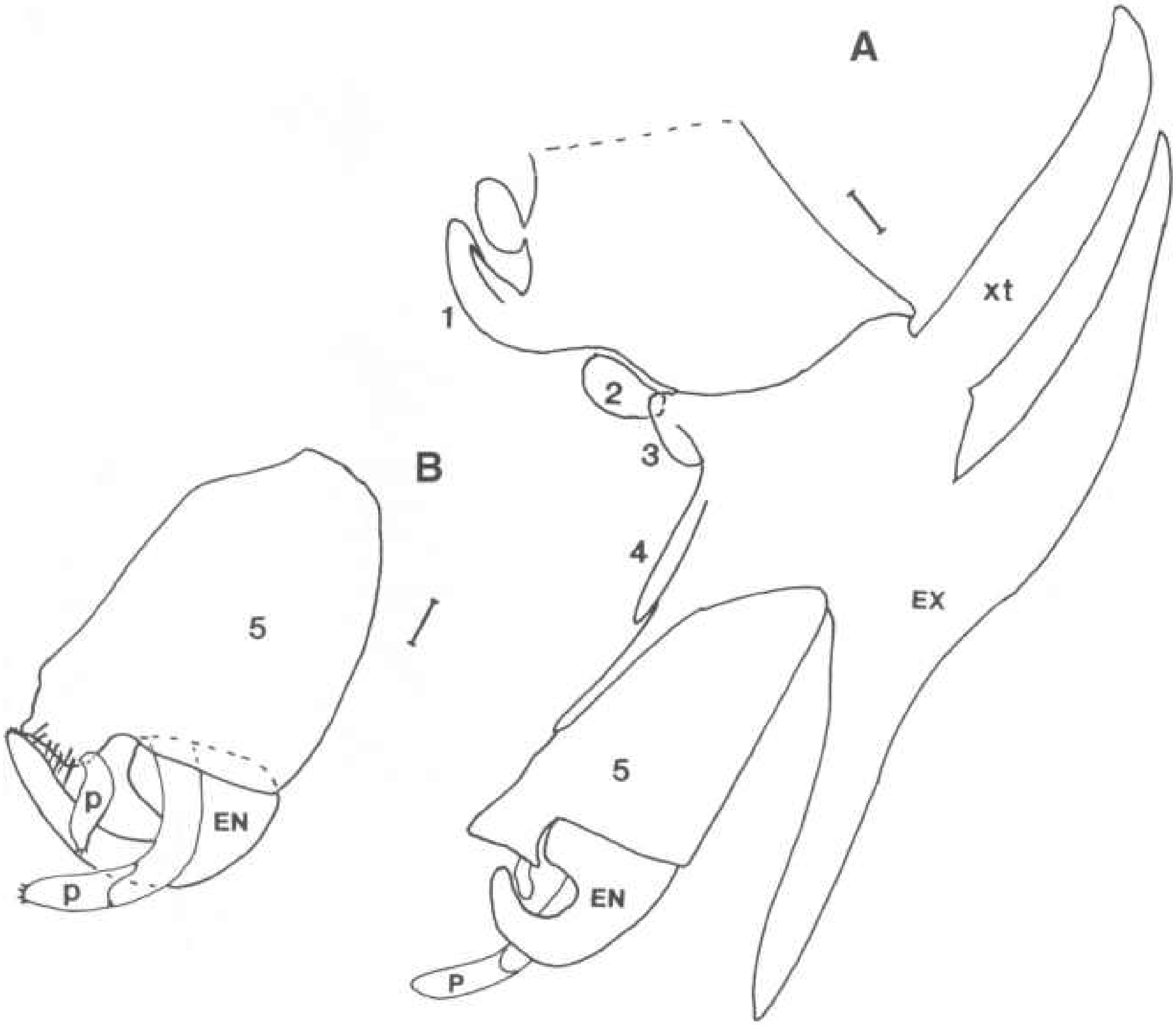

**Figure 4.** *Caenestheriella gifuensis* adult male (7.6 mm × 4.9 mm). A, trunk limb 2, anterior. B, trunk limb 1, distal section, anterior. Abbreviations and symbols as for Fig. 2.

tion; a palp, which articulates at its midlength, originates along the arthrodial membrane separating the proximal and distal sections.

In general, anterior trunk limbs of adult *L. kawachiensis* [Lepestheriidae] are similar to those of adult *C. gifuensis* [Cyzicidae], although trunk limb 1 lacks a posterior knob covered with denticles at the base of the exopod and the proximal lobe of the exopod is more elongate. The exopod of posterior trunk limbs of *L. kawachiensis* is not as well developed as in *C. gifuensis* (compare Fig. 3B with Fig. 8D). During development, trunk limb 6 of *L. kawachiensis* does not appear as a stripe in any early nauplius. An asetose limb from a nauplius (0.2 mm × 0.2 mm) has seven lobes arranged in a flattened semicircle (Fig. 5A). The initial setose limb of trunk limb 6 (from a juvenile 0.7 mm × 0.6 mm) has five enditic lobes, two rami, and an exite (Fig. 5B). The part of the limb bearing enditic lobe 1 does not articulate with the remainder of the limb; both the attenuate lobe on enditic lobe 1 and the discoid lobe are well developed. The setae on enditic lobes 2–5 are ventrally polarized. Palps are found only on adult limbs and so would be added to more anterior limbs during the moult from the last juvenile stage.

Trunk limb 13 appears as a transverse stripe in a juvenile (0.8 mm × 0.6 mm). Its asetose limb in a later juvenile (1.0 mm × 0.8 mm) has six lobes arranged in a flattened semicircle (Fig. 6A). The initial setose limb of

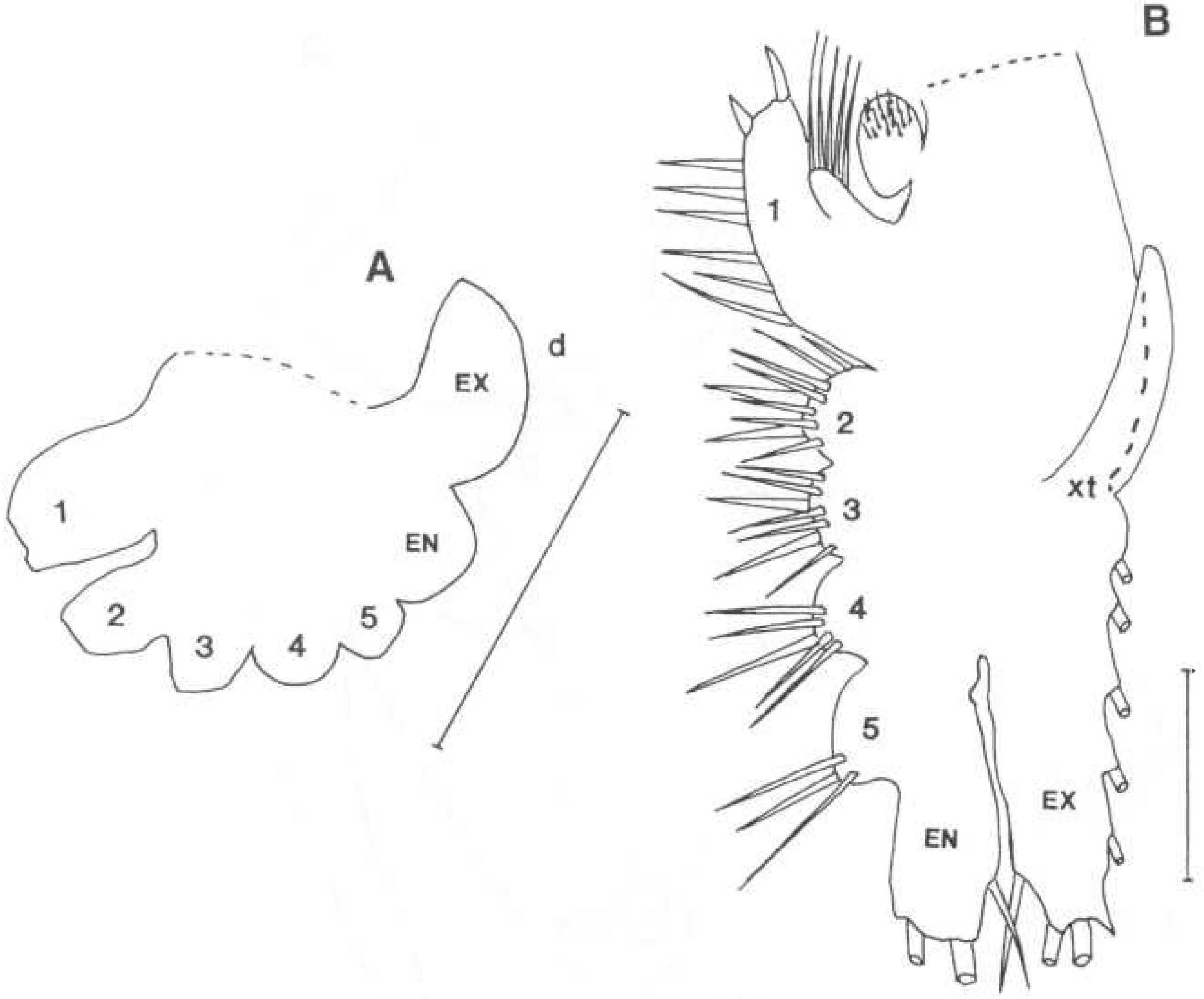

**Figure 5.** *Leptestheria kawachiensis.* A, trunk limb 6, asetose limb, anterior, of nauplius (0.2 mm × 0.2 mm), 'd' is dorsal. B, trunk limb 6, initial setose limb, anterior, of juvenile (0.7 mm × 0.6 mm). Scale bars: 0.05 mm. Abbreviations and symbols as for Fig. 2.

trunk limb 13 (in a juvenile 1.5 mm × 0.8 mm) has five enditic lobes (Fig. 6B); enditic lobe 4 bears one seta, and enditic lobe 5 and the endopod are unarmed. The exopod bears a terminal seta, and an exite is present as a small knob. The part of the limb bearing enditic lobe 1 articulates with the remainder of the limb; a presumptive attenuate lobe is indicated by a seta on the proximal edge of enditic lobe 1 (arrow, Fig. 6B), and the discoid lobe is absent. In the second setose limb (in a juvenile 1.5 mm × 1.0 mm) one seta is added to enditic lobe 5, and several setae are added to the more proximal lobes of this limb. A second seta is added to enditic lobe 5 of the third setose limb (of a juvenile 1.9 mm × 1.3 mm); the endopod remains unarmed (Fig. 6C,D). An unarmed discoid lobe is present in the third setose limb, but the attenuate lobe remains a single seta. The exopod adds a second terminal seta and later two dorsal setae; the exite remains short. Setae on enditic lobes 2–5 in all steps are not polarized.

Trunk limb 19 appears as a transverse stripe in a juvenile (2.0 mm × 1.1 mm). The asetose limb (Fig. 7A) has six lobes arranged linearly (in a juvenile 1.9 mm × 1.3 mm), although trunk limb 18 of the same specimen has all lobes arranged in a flattened semicircle (Fig. 7B). The initial setose limb of trunk limb 19 (Fig. 7C) in a larger juvenile (2.5 mm × 1.6 mm) has five enditic lobes, all armed; enditic lobes 4–5 have one seta each. There is no endopod. The exopod bears two terminal setae and an

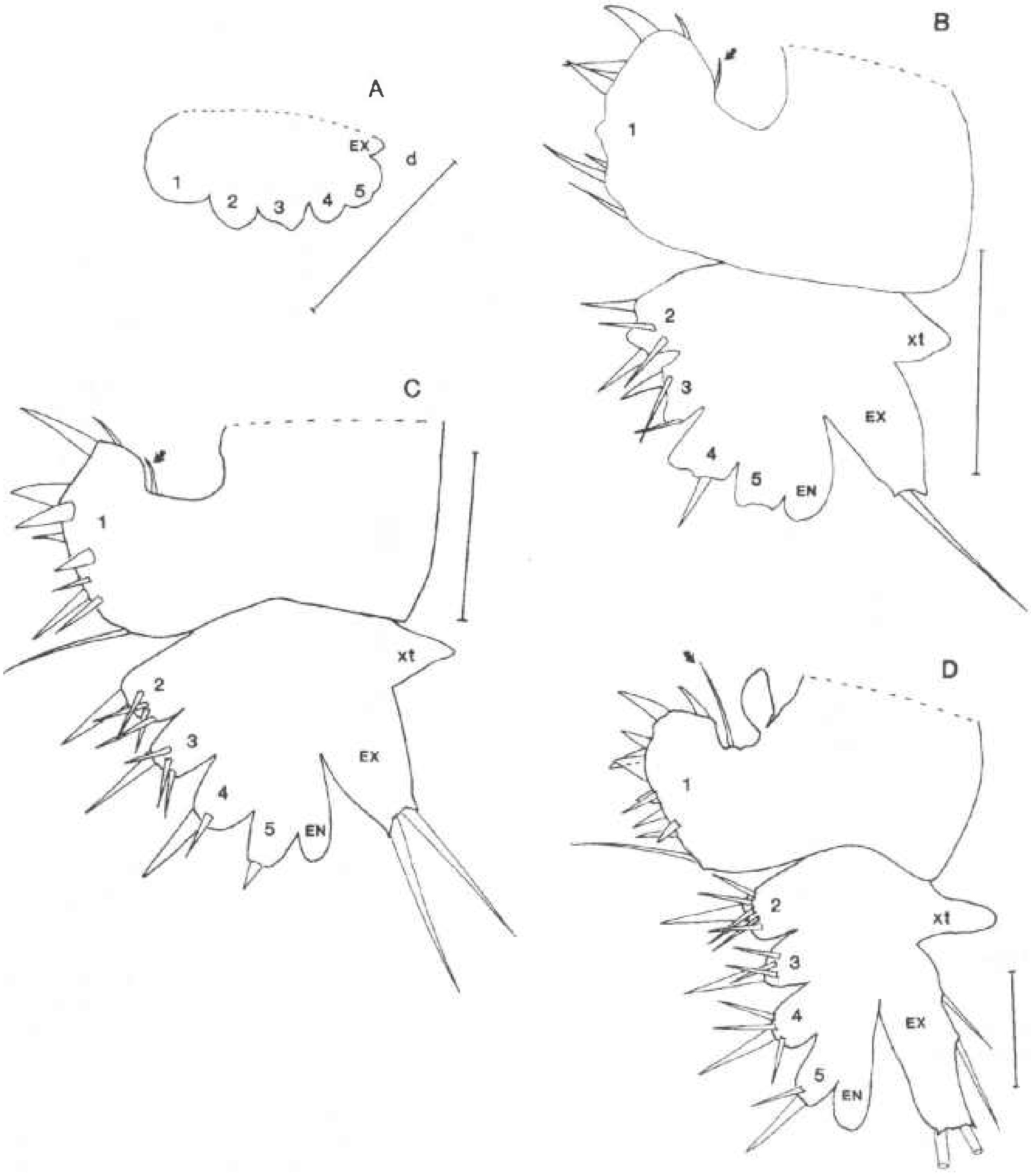

**Figure 6.** *Leptestheria kawachiensis*. A, trunk limb 13 of juvenile (1.0 mm × 0.8 mm), asetose limb, anterior, 'd' is dorsal. B, trunk limb 13 of juvenile (1.5 mm × 0.8 mm), initial setose limb, anterior. C, trunk limb 13 of juvenile (1.5 mm × 1.0 mm), second setose limb, anterior. D, trunk limb 13 of juvenile (1.9 mm × 1.3 mm), third setose limb, anterior. Arrow on seta of presumptive attenuate lobe; remaining abbreviations and symbols as for Fig. 2.

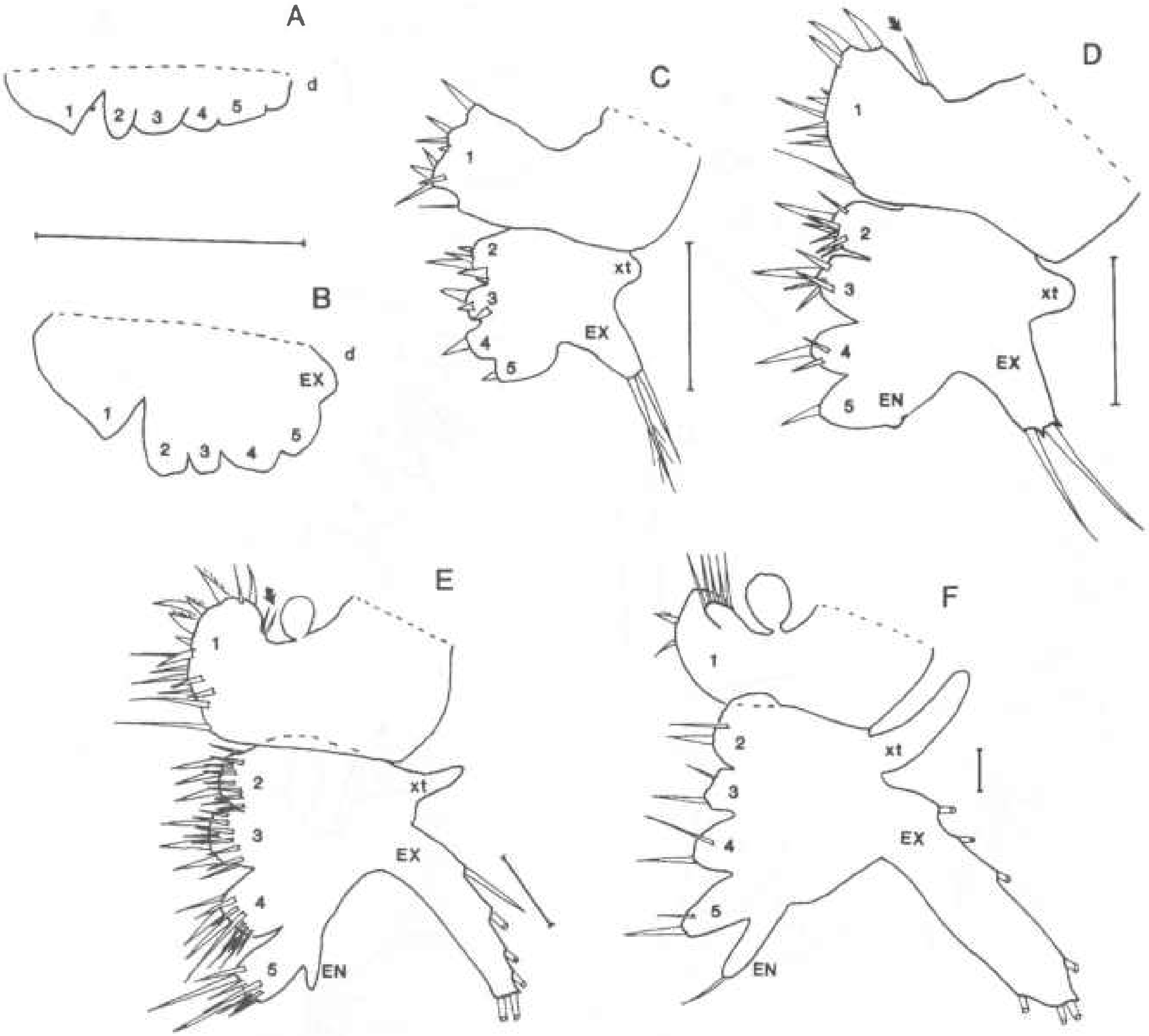

**Figure 7.** *Leptestheria kawachiensis*. A, trunk limb 19 of juvenile (1.9 mm × 1.3 mm), asetose limb, anterior, 'd' is dorsal. B, trunk limb 18 of juvenile (1.9 mm × 1.3 mm), asetose limb, anterior, 'd' is dorsal. C, trunk limb 19 of juvenile (2.5 mm × 1.6 mm), initial setose limb, anterior. D, trunk limb 19 of juvenile (2.8 mm × 1.7 mm), second setose limb, anterior. E, trunk limb 19 of adult (3.9 mm × 2.3 mm), third setose limb, anterior. F, trunk limb 19 of adult (8.0 mm × 4.7 mm), fourth setose limb, anterior; setae on enditic lobes 1–5 omitted. Abbreviations and symbols as for Fig. 6.

exite is present as a small knob. The part of the limb bearing enditic lobe 1 articulates with the remainder of the limb, but there is no seta representing a presumptive attenuate lobe on enditic lobe 1 and no discoid lobe. Setae are added to the more proximal lobes in the following three setose limbs (in a juvenile 2.8 mm × 1.7 mm, an adult 3.9 mm × 2.3 mm, and an adult 8.0 mm × 4.7 mm). The endopod is first indicated by a slight bump near the base of enditic lobe 5; it becomes progressively longer, and is armed with one seta on the oldest examined limb (Fig. 7D–F). The exopod adds dorsal setae in the third setose limb, and then a distal seta and a ventral seta on the fourth setose limb. The exite becomes progressively longer during these three steps of limb development. The part of the limb bearing enditic lobe 1 articulates with the remainder of the limb. The presumptive attenuate lobe on enditic lobe 1 is indicated by a seta in the second setose limb; a second seta is added in the third setose limb, and in the fourth setose limb an attenuate lobe is well developed with five setae. An unarmed discoid lobe is present in the third setose

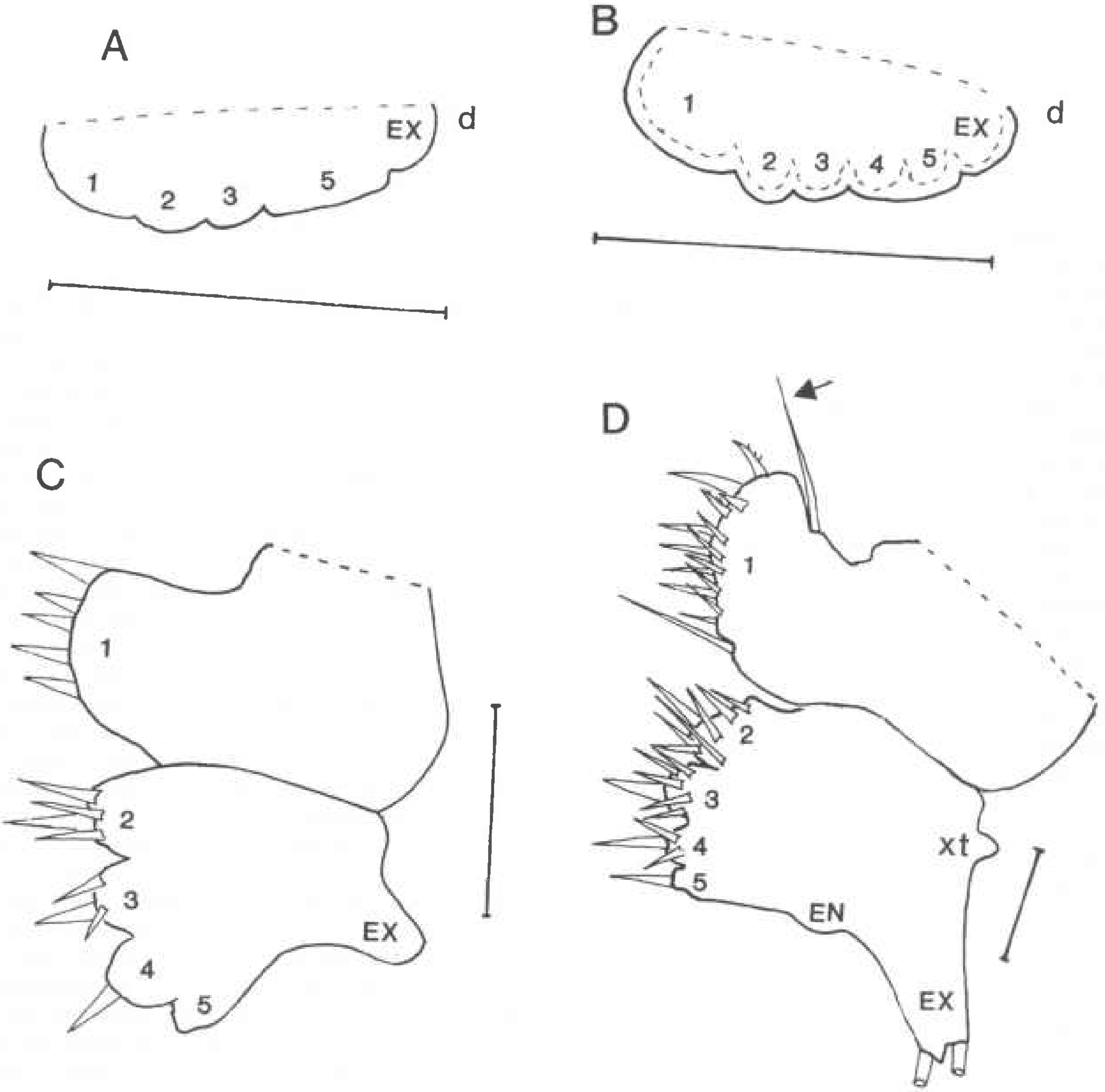

**Figure 8.** *Leptestheria kawachiensis*. A, trunk limb 24 of juvenile (2.3 mm × 1.4 mm), asetose limb, anterior, 'd' is dorsal. B, trunk limb 23 of juvenile (2.3 mm × 1.4 mm), asetose limb, anterior, 'd' is dorsal. C, trunk limb 24 of adult (3.9 mm × 2.3 mm), initial setose limb, anterior. D, trunk limb 24 of adult (8.0 mm × 4.7 mm), second setose limb, anterior. Abbreviations and symbols as for Fig. 6.

limb. The setae on enditic lobes 2–5 remain unpolarized ventrally.

Trunk limb 24 did not appear as a stripe on any specimen here but may have been missed during dissections. Its asetose limb (Fig. 8A) has five lobes arranged linearly (in a juvenile 2.3 mm × 1.6 mm); the second lobe from the distalmost is quite broad. The form of trunk limb 23 from the same specimen (Fig. 7B) suggests that this broad lobe will split into two lobes following the next moult. The initial setose limb of trunk limb 24 (Fig. 8C) in an adult (3.9 mm × 2.3 mm) has six lobes, the proximal three being armed. There is no endopod or exite. The part of the limb bearing enditic lobe 1 articulates with the remainder of the limb. There is no seta marking a presumptive attenuate lobe and no discoid lobe. Setae are added on all lobes of the second setose limb from a larger adult (8.0 mm × 4.7 mm). The endopod and exite are each indicated by a slight bump, and the exopod has two terminal setae. The part of the limb bearing enditic lobe 1 articulates with the remainder of the limb. An attenuate lobe is not developed on enditic lobe 1, but its seta is present. A small bump at the base of the limb may be the presumptive discoid lobe. The setae on enditic lobes 2–5 remain unpolarized ventrally.

## DISCUSSION

### ADULT MORPHOLOGY

To our knowledge, structures corresponding to the attenuate lobe and the discoid lobe of *C. gifuensis* and *L. kawachiensis* have not been reported on a trunk limb of any spinicaudatan. However, Martin & Christiansen (1995) illustrate part of an attenuate lobe for *Leptestheria* sp. with longer setae, and Shakoori (1968) appears to consider the attenuate lobe of *Caenestheria propinquua* (Sars) as the posterior face of enditic lobe 1. In the haplopodan *Leptodora kindtii* (Focke), a knob-like structure covered with tiny spines is associated with the base of only the first pair of legs. This structure has been referred to as a 'processus maxillaris' by Sebestyé (1931: 161) or as a medially displaced endite by Olesen, Richter & Scholtz (2001: 872). In the present study, the discoid lobe was easily missed or destroyed during dissections owing to its proximal position; its rate of recovery from all dissected limbs was less than 50%. An arthrodial membrane separating the proximal part of a trunk limb, bearing enditic lobe 1, from the remainder of the limb has been reported previously for the spinicaudatan *Estheria dahalacensis* Rueppell (e.g. Hansen, 1925: 28; pl. 1, fig. 3a) (*Leptestheria dahalacensis*).

Simple inspection of trunk limbs 13, 19, and 24 of *C. gifuensis* indicates that adult posterior limbs are not miniature adult anterior limbs. Differences in posterior trunk limbs (Table 3) include: absence of a palp; the proximal part of the limb bearing enditic lobe 1 articulates with the remainder of the limb; enditic lobe 1 is not bent proximally; the attenuate lobe of enditic lobe 1 is not well defined; the discoid lobe is a small knob; and the endopod is not well separated from enditic lobe 5.

Larger specimens of both *C. gifuensis* and *L. kawachiensis* show size dimorphism and differences in morphology (e.g. compare Fig. 7E and Fig. 7F) and number of setae (e.g. compare Fig. 8C and Fig. 8D) for the posterior trunk limbs of both sexes. Both size morphs of both genders have 24 setose trunk limbs, no asetose trunk limbs, and no trunk limb stripes (i.e. presumptive limb buds). The dimorphic females with eggs and dimorphic males with transformed trunk limbs 1 and 2 are presumably all adults. Setae of a post-moult limb can be observed within both morphs of both sexes. Adult size polymorphism has been reported in another leptestheriid (Petrov & Marincek, 1995), and we concur with Tasch (1969) that there is no terminal adult moult and possibly no terminal moult for these spinicaudatans. The alternate hypothesis, that populations of both species maintain permanent, size-dimorphic, terminal adults is not supported by the formation of setae of a post-moult limb.

### DEVELOPMENTAL MORPHOLOGY

Among the examined specimens of *L. kawachiensis*, neither carapace dimensions nor number of rings of the carapace could be used to predict developmental stage, as indicted by the number of setose trunk limbs. In discussing the development of these trunk limbs, we assume that no step of limb development was missed, particularly early in development among nauplii without setose trunk limbs (carapace absent or 0.1 mm × 0.3 mm), nauplii with five pairs of setose trunk limbs (0.2 mm × 0.2 mm or 0.3 mm × 0.3 mm), or nauplii with nine pairs of setose trunk limbs (0.7 mm × 0.6 mm). We also assume that each pair of trunk limbs goes through only one asetose-limb step during development, as reported by Anderson (1967: fig. 9). The asetose limb of trunk limb 6 has seven lobes, which correspond to the five enditic lobes and two rami present in the subsequent initial setose limb; the exite is added in this latter step. The asetose limb of both trunk limbs 13 and 19 has six lobes, corresponding to the five enditic lobes and the exopod of the initial setose limb; the exite and endopod are added to this or to the second setose limb. The asetose limb of trunk limb 24 has five lobes, corresponding to enditic lobes 1–3, presumably enditic lobe 5 (see below), and the exopod of the initial setose limb. Enditic lobe 4 is added in this latter step; the endopod and an exite are added as simple knobs to the second setose limb. During development of the trunk limbs of *L. kawachiensis*, the discoid lobe appears before the attenuate lobe, but after the setae of the presumptive attenuate lobe have appeared. Palps are added during the moult from the last juvenile stage; palps of *L. kawachiensis* did not become segmented during adult development, as has been reported for *L. saetosa* Petrov & Marincek by Petrov & Marincek (1995).

These observations suggest that the development of anterior trunk limbs, like trunk limb 6, is accelerated relative to that of more posterior trunk limbs 13, 19, and 24. The asetose limb of trunk limb 6 has one more lobe, corresponding to the endopod, than the same limb of trunk limbs 13 and 19, and two more lobes, corresponding to the endopod and enditic lobe 4, than that of trunk limb 24. The missing lobes of the posterior limbs are added later in development to one of the setose limbs. All of the enditic lobes of the initial setose limb of trunk limb 6 have setae and all of the setae are polarized ventrally; only enditic lobes 1–3 of the initial setose limb of trunk limb 24 have setae but these setae are not polarized ventrally. The question of truncation of development of posterior trunk limbs also is clear. Development of trunk limbs 19 and 24 is truncated relative to that of trunk limb 13. The endopod of trunk limbs 19 and 24 does not bear more than one seta on the largest specimen examined, whereas the endopod of trunk limb 13 of the same specimen has many setae. The arthrodial membrane separating the part of the limb bearing enditic lobe 1 from the remainder of the limb is retained on trunk limbs 19 and 24 of this specimen, whereas this arthrodial membrane is absent on trunk limb 13.

### LIMB PATTERNING

Enditic lobes 4–5 of *L. kawachiensis* are formed within the same pre-existing lobe of the asetose limb of trunk limb 23. Later, the endopod of trunk limbs 13, 19, and 24 is formed distally to enditic lobe 5. In addition, enditic lobes 4–5 of trunk limb 24 of adult *C. gifuensis* are poorly differentiated. We assume simply that one section of the distal part of the limb, enditic lobe 5, patterns enditic lobe 4 proximally and the endopod distally. Alternative, more complex explanations would include more than one patterning location, but the simpler, single patterning centre is preferred here, like that of the copepod maxilliped (Ferrari, 1995). Support for the conclusion that the endopod develops from or is patterned by enditic lobe 5 also is found in the development of *Cyclestheria hislopi* (Baird) [Cyclestheriidae], a species whose distinctness has led some authors (e.g. Olesen *et al.*, 1996; Olesen, 1998) not to include it among the spinicaudatans. Olesen (1999: 174, fig. 10D) shows that the endopod of the trunk limb of *C. hislopi* splits from enditic lobe 5. The origin of the endopod of *C. hislopi* and *L. kawachiensis* takes place later than that of the proximal endopodal segment, a situation that is reversed for the copepod maxilliped. Among copepod thoracopods, development of the distal segment of the endopod, represented by the two terminal setae on the limb bud, occurs during the transformation of the limb (Ferrari, 2000; Ferrari & Ivanenko, 2001). Patterning of the proximal segments of the endopod occurs later, after the transformation.

Patterning of the proximal section of the spinicaudatan trunk limb is incomplete and so less clear. Some setae of the attenuate lobe appear before the discoid lobe but after enditic lobe 1; the discoid lobe is formed last. The proximal section of the limb, then, appears to be patterned distally to proximally. This patterning mirrors, i.e. reverses, the loss of setose lobes proximally to distally on the praecoxa of the maxilliped among derived cyclopoid copepods (Ferrari & Ivanenko, 2001). Ancestral cyclopinids and oithonids have the middle and distal setose lobes on the praecoxa. Some cyclopids have the distal setose lobe on the praecoxa, whereas derived cyclopids lack setal lobes on the praecoxa.

### SEGMENT HOMOLOGIES

In order to determine homologies of the distal part of these spinicaudatan trunk limbs (Fig. 9A), we compare their development to that of the maxilliped of copepods, which is the only crustacean trunk limb whose patterning during development has been well studied (Ferrari, 1995; Ferrari & Dahms, 1998; Ferrari & Ivanenko, 2001). Comparable data from other branchiopods, living or fossil, unfortunately are not available. From the data on spinicaudatan patterning, we conclude that enditic lobe 5 is homologous to the segment of the copepod maxilliped that patterns its endopod, namely the proximal endopodal segment of the copepod maxilliped observed at the first copepodid stage. Enditic lobe 4 of the spinicaudatan is thus homologous to the proximal endopodal segment of the maxilliped of later copepodid stages of many copepods. Finally, the traditional endopod of the spinicaudatan is homologous only to the distal part of the terminal segmental complex of the endopod of the copepod maxilliped (Ferrari & Ivanenko, 2001: fig. 15). Enditic lobe 5 patterns only two lobes on the female trunk limb; the endopod, then, is assumed to be three-segmented (Fig. 9A), in agreement with Hansen (1925: 24, 29), who re-interpreted Huxley's (1877) study of a notostracan to infer conchostracan endopodal segmentation.

Homologies of the spinicaudatan female protopod are suggested by the presence of an arthrodial membrane between the proximal part of the limb bearing enditic lobe 1 and the remainder of the limb, and the origin of the exite distal to this arthrodial membrane, as well as the presence of a single coxal lobe on the copepod maxilliped. The homologies preferred here are that enditic lobe 3 of the spinicaudatans (Fig. 9A) is homologous to the lobe of the basis of the maxilliped of cyclopoid copepods (Ferrari & Ivanenko, 2001). Enditic lobe 2 is the coxal lobe of the limb. Enditic lobe 1, the attenuate lobe, and the proximal, discoid lobe are homologous to the three praecoxal lobes of the calanoid copepod maxilliped; the proximal lobe of the three is absent on the cyclopoid copepod maxilliped (Ferrari & Ivanenko, 2001). This hypothesis is in general agreement with Hansen's (1925: 29) interpretation of the sympod of *Estheria dahalacensis* (now *Leptestheria dahalacensis*) or *Limnetis brachyura* Mueller (now *Lyncaeus brachyura*). The spinicaudatan exite, then, is an epipod because it originates on the coxa. An different hypothesis, that the part of the trunk limb proximal to the arthrodial membrane is a syncoxa and the basis has two lobes, is not preferred

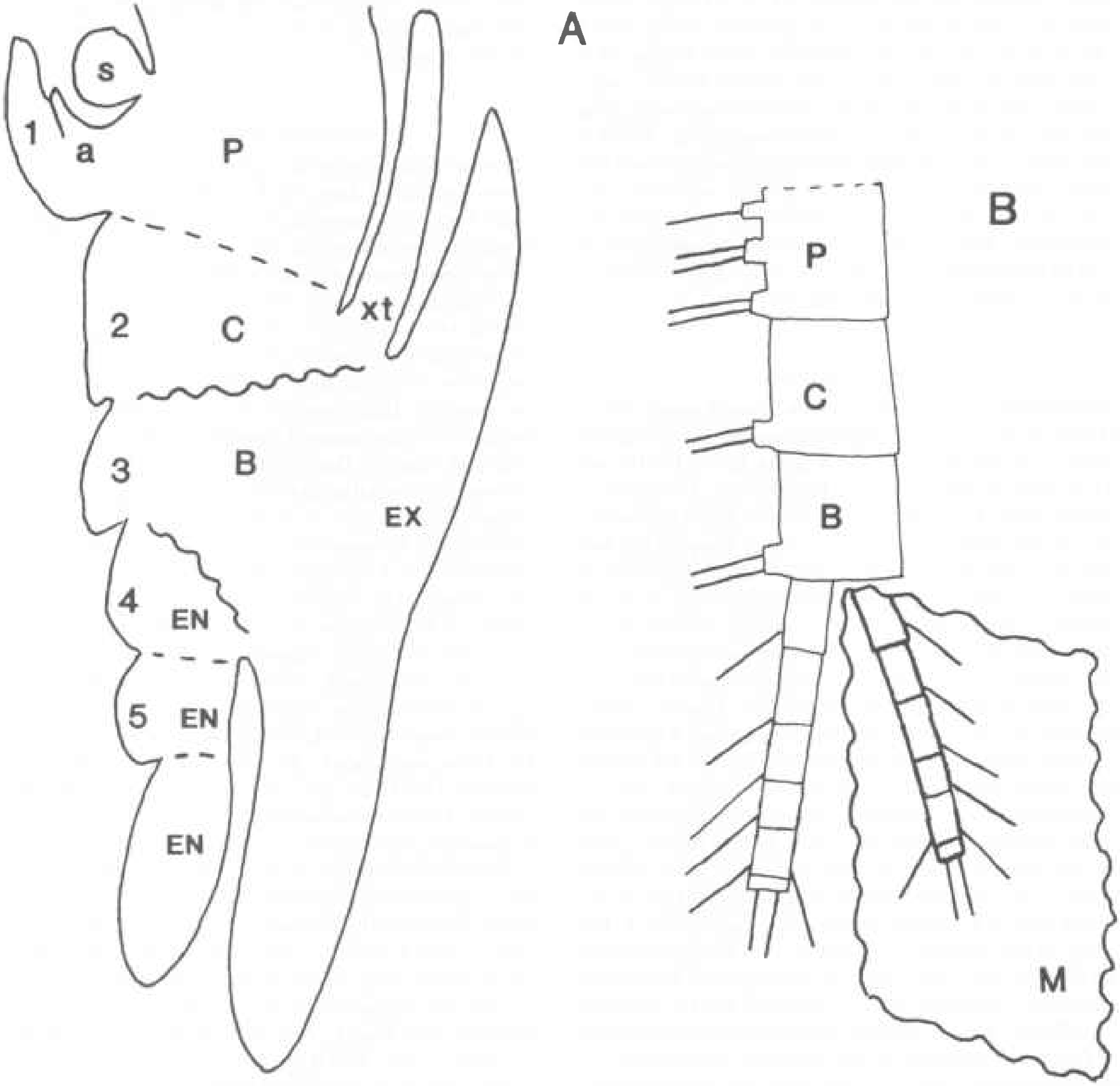

**Figure 9.** A, outline of trunk limb 13 of adult female of *Caenestheriella gifuensis* showing inferred position of praecoxa (P), coxa (C), basis (B), exite (xt), exopod (EX), and the three segments of endopod (EN). Enditic lobes 1–5 are numbered; a, attenuate lobe; s, discoid lobe; broken line proximal to coxa represents arthrodial membrane present in younger limbs; broken line proximal to distal and middle endopodal segments represents arthrodial membranes present in transformed limbs of male; wavy lines are presumed locations of boundaries between proximal endopodal segment and basis, and between basis and coxa. B, hypothetical reconstruction of the origin of the biramal limb by axis duplication. Adult limb with setae along the duplicated ramus (exopod in thick lines) located dorsally and terminally, but only distad along the ventral face; the positions are a mirror image of the setae along the original ramus (endopod in thin lines), which are ventral and terminal, but only distad along the dorsal face; six endopodal segments from Siewing (1960); shape of segments is not significant. Praecoxa (P), coxa (C), basis (B); wavy line with M is mirror tilted slightly upward, toward the viewer.

because the exite of the spinicaudatan must then be interpreted as a pseudepipod. A further hypothesis, that the part of the trunk limb proximal to the arthrodial membrane is a coxa, is not preferred because this coxa has three lobes, rather than one on the copepod maxilliped, and again the exite of the spinicaudatan becomes a pseudepipod. Our interpretation of homologies of the trunk limbs of the present spinicaudatans, based on comparative developmental morphology, contradicts interpretations based on comparative adult morphology of Snodgrass (1956:72) 'six endites represent coxopodite through protopodite' and Walossek (1993: 63) 'a small "proximal endite" and a large "sympodite" which is nothing more than the retained but enlarged basipod carrying the rami . . .'.

Difficulty in determining homologies of the transformed male trunk limbs 1 and 2 of spinicaudatans with the corresponding female trunk limbs was noted most recently by Olesen *et al.* (1996). Problems in understanding homologies of the male limbs in previous studies (e.g. Botnariuc, 1947; Shakoori, 1968; Petrov & Marincek, 1995) may result from an underestimate of the number of lobes present in the poorly sclerotized middle part of the limb. In our study, the morphology of the well-sclerotized proximal part of transformed male trunk limbs 1–2 of *C. gifuensis* corresponds to that part of the female limb bearing enditic lobe 1, the attenuate lobe, and the discoid lobe. The poorly sclerotized middle part bears enditic lobes 2–4; they appear distorted owing to contraction of limb muscles. The well-sclerotized distal part consists of a broad, proximal section, articulating proximally and distally, and a curved, finger-like, distal section. The proximal section with its palp is homologous to enditic lobe 5 of the female trunk limb, and corresponds to the middle endopodal segment. The distal section is homologous to the traditional endopod of the female trunk limb (i.e. the third endopodal segment). The articulating palp, originating between the proximal and distal segments, is not homologous to any structure on the female trunk limb. Its location is immediately distal to enditic lobe 5, from which both enditic lobe 4 and the traditional endopod develop. This location suggests the articulating palp may be an incomplete fourth endopodal segment, distally patterned from enditic lobe 5 during the transformation of the male limb, in the same manner that enditic lobe 4 and the traditional endopod are patterned.

## GENE EXPRESSION AND MORPHOLOGICAL HOMOLOGIES

Studies to date that have applied gene expression data to the comparative morphology of arthropod limbs have tried to identify homologous parts of corresponding limbs in different branchiopods but have not tried to clarify the homologies with limb parts in other crustaceans (e.g. Olesen *et al.*, 2001), or have tried to point out the difficulty in establishing such homologies (Williams, 1999). One problem with determining segmental homologies of arthropod limbs from the expression patterns of regulatory genes during development is that there is no generally accepted or clearly defined model indicating at what point in ontogeny an expression pattern can be interpreted as a morphological homology. In suggesting that the wing of an insect is homologous to the coxal exite of a branchiopod, Averof & Cohen (1997) used a later and more restricted expression of proteins of the genes *apterous* and *nubbin* than that seen earlier in development when the proteins are expressed throughout the limb of the brine shrimp and in several places in the fly. However, problems arise in applying a restricted, late-expression model to morphological homologies corresponding to the protein expression of *distal-less*, a gene that is required for development of the fruitfly thoracopod. *distal-less* protein is expressed throughout the thoracopod area of the imaginal disc early in development in the fruitfly (Cohen & Juergens, 1989), as well as throughout the antenna 2 and mandible of naupliar brine shrimp (Panganiban *et al.*, 1995; our interpretation of their fig. 4A and personal observations of F.D. Ferrari). Later, *distal-less* expression becomes restricted to an area that will become the telopod of the fruitfly thoracopod (González-Crespo & Morata, 1995), namely the femur through the pretarsus, which is homologous to the endopod of crustaceans (Paulus, 1996: abb. 596). Still later in development, *distal-less* expression is restricted to the presumptive distal tibia, tarsus, and pretarsus. Finally, at the third instar, a secondary ring of expression in the area that will become the proximal boundary of the femur with the trochanter (the proximal boundary of the ischium with the basis of malacostracan crustaceans, or the proximal boundary of the ramus with the basis in copepods) expands the area of expression (Wu & Cohen, 1999). It is not clear whether the most restricted but earlier pattern, or the later but expanded pattern, should be regarded as defining morphological homologies in thoracopods of other arthropods.

## MULTIBRANCHED TRUNK LIMBS

Recent studies have described branchiopod trunk limbs as multibranched (Nulsen & Negy, 1999; Williams, 1999), apparently based on the morphological similarities of the lobe-like endites and the rami early in development. The following structures of the branchiopod trunk limb, however, have distinct and separate identities: an endite, which is a non-articulating ventral attenuation of a protopodal segment; an exite, which is an articulating, dorsal elongation, of unknown axial orientation, originating on a protopodal segment; a ramus, which may be composed of a group of articulating, serially homologous elements along a presumably proximodistal axis originating on the distal protopodal segment; and a palp, which is a ventral elongation, articulating in some cases, of unknown axial orientation and originating on an endopodal segment. The absence of arthrodial membranes may obscure the serially homologous nature of a particular ramus, but this condition is not enough to justify identifying rami with endites or exites.

## EVOLUTIONARY TRANSFORMATIONS OF TRUNK LIMBS

Contrary to Calman (1909: 51), Borradaile (1926), or Fryer (1992, 1996), we do not find the poorly sclerotized, flattened limbs of female spinicaudatans to be attributes that suggest this morphology is necessarily primitive for branchiopods. Younger trunk limbs in females are sclerotized proximally with an arthrodial membrane separating two parts of the limb. Both a proximal and a distal part of transformed male trunk limbs 1 and 2 are sclerotized. Copepod swimming legs are usually well-sclerotized trunk limbs that also are flattened anterioposteriorly. The presence of ventral setae on the exopod of the copepod swimming leg throughout development (Ferrari & Benforado, 1998; Ferrari, 2000), and of a coupler uniting the contralateral limb pairs, suggests that copepod swimming legs are not primitive but derived thoracopods, relative to the homologous limb pairs of a more basal crustacean ancestor. Ventral setation on the exopod and dorsal setation on the endopod, numerous setae on the enditic lobes, the specific, repeated pattern of those setae on lobes 2–5, as well as flattening and poor sclerotization, suggest that spinicaudatan trunk limbs also may have been derived, perhaps from a stenopodal, or at least a more clearly segmented, ancestral morphology.

A particularly clear example showing that a phyllopodal morphology may be derived from a stenopodal limb is provided by the maxilliped of the harpacticoid copepods *Longipedia americana* Wells or *Coullana canadensis* (Willey), both of which lack arthrodial membranes and are flattened anterioposteriorly, suggesting a phyllopod-like morphology. The number and homologies of the presumptive segments of the limb relative to the stenopod-like maxilliped of other copepods can be inferred from the stages of development at which particular setae are added to the limb (Ferrari & Dahms, 1998). Longipediidae and Canuellidae are placed at the base of the Harpacticoida (Lang, 1948), and many of the more derived harpacticoids have well-sclerotized, stenopod-like maxillipeds. In addition, Harpacticoida are considered to be derived copepods (Ho, 1990; Martínez Arbizu, 2003); the older orders Misophrioida and Calanoida also are characterized by well-sclerotized, stenopod-like maxillipeds. These phylogenetic relationships were not derived using characters of the maxilliped; therefore, a stenopod-like morphology can be inferred for the maxilliped of copepods both more primitive and more derived than *L. americana* and *C. canadensis*. We agree, then, with Borradaile (1926) and Olesen *et al.* (2001) that a stenopodal morphology may have been recovered from a phyllopodal morphology during branchiopod evolution, for example in *Leptodora kindtii*, but we also believe that the phyllopodal morphology of spinicaudatans may have been derived from a more stenopodal morphology sometime during the evolution of branchiopods.

## ANCESTRAL CRUSTACEAN THORACOPOD

Although the attributes ‘poorly sclerotized’ and ‘flattened anterio-posteriorly’ may not define an ancestral branchiopod trunk limb, the presence early in spinicaudatan development of an exopod lacking ventral setae is suggestive of a primitive crustacean limb. One model for the evolution of the biramal limb among arthropods is by duplication of the proximodistal axis, described by Diaz-Benjumea & Cohen (1994). If this is the case, then the duplicate rami should be mirror images of each other. That is, the exopod should appear like an image from a mirror placed dorsally to the endopod (Fig. 9B), or the endopod should appear like an image from a mirror placed ventrally to the exopod. In general, setae of the endopods of most crustaceans occur on the terminal face and all along the ventral face, but only distally along the dorsal face. Assuming this distribution is primitive, and an axis duplication model is correct, the primitive crustacean exopod should have setae on the terminal face and all along the dorsal face, but only distally along the ventral face (Fig. 9B). This morphology is similar to that of the third setose limb of trunk limb 13 of *Leptestheria kawachiensis* (Fig. 6D), the third and fourth setose limbs of trunk limb 19 of *L. kawachiensis* (Fig. 7E,F), and trunk limb 24 of adult *Caenestheriella gifuensis* (Fig. 3B), although these exopods, like the endopods, have no arthrodial membranes.

## SUMMARY

The trunk limbs of *C. gifuensis* and *L. kawachiensis* are more complex than might be inferred from the earlier cited studies of spinicaudatan limbs. They are flattened anterioposteriorly and usually poorly sclerotized. There is an anterioposterior trend in the gradual reduction in size among them, but there also are more abrupt changes in structure along the anterior–posterior axis (Table 3), which may be traces of trunk tagmosis of an ancestor. Differences in development of different trunk limbs may reflect either acceleration or truncation, depending upon trunk limb position along the anterior–posterior axis. These complexities are reflected in the general order of patterning of the spinicaudatan trunk limb during development. In females, a protopod of three enditic lobes and the middle endopodal segment form earliest, followed by the proximal endopodal segment and the exopod. The distal endopodal segment, as an unarmed knob, and the exite, as a knob, form next. Initial setation of the presumptive attenuate lobe precedes the formation of the discoid lobe, but elongation of the attenuate lobe occurs later; development of a palp on enditic lobe 5 is last. Male development is similar to that of the female, but the distal articulating palp, which may be a fourth endopodal segment, is last. A lack of sclerotization may obscure the homologies of these limbs, but they are biramal if the exite is not considered a ramus. There is nothing intrinsic to the structure of the adult trunk limbs to suggest that they are similar to the adult limbs of the ancestral branchiopod or the ancestral crustacean.

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