# VARIABILITY OF TRUNK LIMBS ALONG THE ANTERIOR/POSTERIOR BODY AXIS OF JUVENILE AND ADULT *LYNCEUS BIFORMIS* (ISHIKAWA, 1895) (BRANCHIOPODA, LAEVICAUDATA, LYNCEIDAE)

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## ABSTRACT

Morphology of trunk limbs of the laevicaudatan branchiopod *Lynceus biformis* (Ishikawa, 1895) varies along the anterior/posterior axis of the body. On anterior limbs, the praecoxal endite is elongate and strongly flexed, endites of the coxa and basis are broad, and lobes of the endopod are elongate. On more posterior limbs protopodal endites and endopodal lobes are more similar to each other and none is particularly long or broad. In addition, an exite fails to form on more posterior limbs, and the exopod becomes progressively reduced in males or modified in females. Sexual dimorphism is expressed by fewer trunk limbs, 10, and a transformed trunk limb 1 of males, as well as by a modified exopod of posterior trunk limbs 8-12 of females. Development of all limbs includes an asetose step and several setose steps; some limb pairs may begin as a transverse bilobate flap. Developmental changes include the progressive addition of limbs posteriorly, addition of endites and lobes to some limbs, addition of an exite and proximal rod-like structure during the transformation to a setose limb, and the transformation of sexually dimorphic limbs during the molt to the adult. Naupliar asetose limbs 1-5 have fewer endites and lobes than the more posterior asetose limbs of juveniles have.

The male clasper of *L. biformis* is a subchela formed from the basis and the distal segment of the endopod; the proximal and middle endopodal segments are closely associated with the subchela. In contrast, the clasper of male spinicaudatans is a subchela formed from the distal endopodal segment and the proximal of two middle segments of the endopod. The distal of the two middle endopodal segments and the palp it bears (together, the so-called "articulated palp") are absent in the clasper of *L. biformis*. The modified exopod on trunk limbs 7-10 of females of *L. biformis* has no similarly transformed counterpart on limbs of female spinicaudatans. There is no discoid endite on the trunk limb of *L. biformis* as there is on trunk limbs of spinicaudatans, and no setose attenuate endite either, although the asetose rod-like structure may be homologous to the latter.

## ZUSAMMENFASSUNG

Der Bau der Beine des Rumpfes von *Lynceus biformis* (Ishikawa, 1895) (Branchiopoda, Laevicaudata) variiert von vorn nach hinten entlang der Längsachse. Vordere Beine haben einen länglichen

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Also available online: www.brill.nl/cr
Crustaceana 85 (3): 359-377
DOI:10.1163/156854012X624655

und stark gebogenen Präcoxalenditen, breite Enditen an Coxa und Basis sowie längliche Endopodiallappen. Weiter hinten gelegene Beine haben Protopodialenditen und Endopodiallappen, die einander ähnlicher sind. Keiner ist besonders lang oder breit. Außerdem fehlt ein Exit an den weiter hinten gelegenen Beinen und der Exopodit wird bei den Männchen zunehmend reduziert und bei den Weibchen modifiziert. Sexualdimorphismus findet seinen Ausdruck in weniger Beinen des Rumpfes (10), einem abgewandelten ersten Rumpfbein bei den Männchen und in einem modifizierten Exopoditen an den Beinen 8-12 des Rumpfes der Weibchen. Die Entwicklung aller Beine durchläuft ein Stadium ohne Borsten und mehrere Stadien mit Borsten; einige Beinpaare beginnen als transversale, zweilappige Anhänge. Veränderungen während der Entwicklung bestehen in der Zunahme hinterer Beine, dem Auftreten von Enditen und Loben an einigen Beinen, der Hinzufügung eines Exiten und einer stabartigen proximalen Struktur während der Umwandlung in ein Bein mit Borsten und in der Umwandlung der sexualdimorphen Beine während der Erwachsenenhäutung. Die borstenlosen Beine 1-5 des Nauplius haben weniger Enditen und Loben als die weiter hinten gelegenen borstenlosen Beine juveniler Exemplare.

Das männliche Greiforgan von *L. biformis* ist eine Subchela , die aus der Basis und dem distalen Glied des Endopoditen besteht; das proximale und das mittlere Endopoditenglied sind eng mit der Subchela verbunden. Im Gegensatz dazu ist das männliche Greiforgan der Spinicaudata eine Subchela, die vom distalen Endopoditenglied und dem proximalen der beiden mittleren Glieder gebildet wird. Das distale der beiden mittleren Endopoditenglieder mit dem von ihm getragenen Palpus (zusammen der sogenannte „gegliederte Palpus“) fehlt dem Greiforgan von *L. biformis*. Der modifizierte Exopodit der Beine 7-10 des Rumpfes der Weibchen von *L. biformis* ist ohne vergleichbares Gegenstück bei den Weibchen der Spinicaudata. Anders als bei den Spinicaudata sind die Beine des Rumpfes von *L. biformis* ohne scheibenförmigen Enditen und auch ein verkleinerter beborsteter Endit ist nicht vorhanden, wiewohl die stabförmige borstenlose Struktur mit ihm homolog sein könnte.

## INTRODUCTION

*Lynceus biformis* (Ishikawa, 1895) is a laevicaudatan clam shrimp first described as *Limnetis biformis* from a lotus paddy in Tokyo and rice paddies in what are today southern Ibaraki and western Gifu Prefectures, Japan (Ishikawa, 1895). Since then, it has been recorded only from rice paddies in the main island of Honshu, ranging from Yamagata and Miyagi Prefectures in the north to Hyogo Prefecture in the southwest (Kasumi, 1961; Igarashi, 1966; Sekiguchi, 1978; Grygier et al., 2002; Ikezawa, 2005; Ishida & Yamanishi, 2006; Kuno, 2007; Ishida, 2010; Grygier, 2011; M. J. Grygier, unpubl. data). It also has been reported from rice paddies in South Korea (Yoon & Kim, 2000) and an ephemeral pool in Taiwan (Wang et al., in press). Light microscopical and SEM photos of the nauplius larvae have been published (Lin & Chou, 1991, fig. 13; Olesen, 2007, fig. 5D), but no full description of the larval development has yet appeared. Females from Korea reportedly have only 10 pairs of setose trunk limbs (Yoon & Kim, 2000). However, our specimens from Ha-dong, Gyeongju-si, Gyeongsangbuk-do, South Korea have 12 pairs of setose trunk limbs, like females from Japan (see below).

Research on the trunk limbs of laevicaudatans has been a topic of interest among carcinologists. Martin et al. (1986) documented changes in trunk limbs along the anterior/posterior axis of the body for males of *L. gracilicornis* (Packard, 1871) and Olesen (2005) described aspects of trunk limb morphology of developmental stages of *L. brachyurus* Müller, 1776. Fryer & Boxshall (2009) proposed from morphology that the trunk limbs of *L. gracilicornis* and *L. simiaefacies* Harding, 1941 are used to collect particulate detritus by scraping or sweeping such material from surfaces with modified setae that they called scrapers and scooping spines.

Here we describe changes in morphology of the protopodal endites, endopodal lobes, exopod and exite of trunk limbs along the anterior/posterior axis of the body of naupliar, juvenile and adult specimens of both sexes of *L. biformis* collected from rice paddies in Japan. Until now, the only detailed study of any appendage in this species has been an SEM-based description of the gnathal edge of the mandible (Richter, 2004). Results of the present analysis are compared with our earlier, detailed study of changes in trunk limb morphology during post-embryonic development of two spinicaudatan branchiopods, *Caenestheriella gifuensis* (Ishikawa, 1895) and *Leptestheria kawachiensis* Uéno, 1927 (see Ferrari & Grygier, 2003).

## SPECIMENS AND METHODS

Specimens of *Lynceus biformis* were collected from several rice paddies in Kataoka-cho, Kusatsu City, Shiga Prefecture, Japan using a conical dip-net of 15 cm diameter and depth, and 65 $\mu$m mesh. Sampling was conducted in two similar but distinct environments at different times of year: (1) paddies recently flooded with irrigation water from a canal at the beginning of the planting season (24 and 26 May 2004, 8-15 May 2005: juveniles preserved in 70-80% ethanol), and (2) rainwater puddles formed in ruts in paddies following the harvest (19-29 September 2002: nauplii and juveniles preserved in 5% formalin; 31 October 2002: adult females and males preserved in 80% ethanol). Nauplii and small juveniles were taken by swishing the dip-net back and forth several times, thus stirring up the bottom. Later they were isolated alive from other plankton, microbenthos, and detritus under a dissecting microscope, but specimens larger than about 2 mm diameter (i.e., big enough to see by naked eye) were caught one-by-one on site. Laboratory rearing was not conducted.

Specimens from these samples were subsequently sorted by carapace size and then by the number of trunk limbs. The examined specimens of *L. biformis* are catalogued as USNM 1159093. [N.B.: References to spincaudatans here direct to the Ferrari & Grygier (2003) study of *Caenestheriella gifuensis* (USNM 1159091) and *Leptestheria kawachiensis* (USNM 1159092).]

Adult females of *L. biformis*, with 12-13 pairs of trunk limbs (including the most posterior pair, expressed as a bilobate flap), were identified by the presence of eggs

TABLE I

Size and trunk limb configurations of examined specimens of *Lynceus biformis* (Ishikawa, 1895). a = carapace length (anterior to posterior) in mm; b = carapace width (dorsal to ventral) in mm; c = number of pairs of setose trunk limbs; d = number of pairs of asetose trunk limbs; e = number of transverse posterior bilobate flaps; f = sex or life-history phase; g = specimen number

| a | b | c | d | e | f | g |
|---|---|---|---|---|---|---|
| 5.03 | 4.47 | 12 | 0 | 1 | female | #18 |
| 2.06 | 1.78 | 12 | 0 | 1 | female | #20 |
| 1.97 | 1.67 | 12 | 0 | 1 | female | #15 |
| 1.57 | 1.33 | 12 | 0 | 1 | female | #13 |
| 1.36 | 1.09 | 11 | 0 | 1 | female | #30 |
| 4.66 | 4.50 | 10 | 0 | 1 | male | #17 |
| 3.47 | 3.25 | 10 | 0 | 1 | male | #21 |
| 2.68 | 2.39 | 10 | 0 | 1 | male | #16 |
| 2.10 | 1.97 | 10 | 0 | 1 | male | #22 |
| 1.83 | 1.58 | 10 | 0 | 1 | male | #23 |
| 1.03 | 0.88 | 10 | 0 | 1 | juvenile | #24 |
| 1.19 | 0.94 | 9 | 1 | 1 | juvenile | #10 |
| 1.00 | 0.88 | 9 | 1 | 1 | juvenile | #29 |
| 1.70 | 1.47 | 9 | 1 | 1 | juvenile | #19 |
| 0.83 | 0.73 | 7 | 2 | 1 | juvenile | #27 |
| 0.78 | 0.68 | 7 | 2 | 1 | juvenile | #28 |
| 0.54 | 0.61 | 7 | 1 | 1 | juvenile | #12 |
| 0.56 | 0.52 | 6 | 1 | 1 | juvenile | #26 |
| 0.60 | 0.51 | 5 | 2 | 1 | juvenile | #14 |
| 0.59 | 0.52 | 5 | 2 | 1 | juvenile | #02 |
| 0.57 | 0.48 | 5 | 2 | 1 | juvenile | #25 |
| 0.46 | 0.45 | 5 | 2 | 1 | juvenile | #11 |
| 0.43 | 0.54 | 0 | 5 | 0 | nauplius | #05 |
| 0.39 | 0.41 | 0 | 5 | 0 | nauplius | #01 |

attached to the inside surface of the valves. Adult males, with 11 pairs of trunk limbs (again including the bilobate flap), were identified by a clasper-like trunk limb 1. A series of juveniles had 8-11 pairs of trunk limbs (including a bilobate flap), and older nauplii had 5 pairs (but no bilobate flap).

Carapace size and trunk limb numbers by kind — setose and asetose limb pairs, and bilobate flap — are given in table I for each of the 24 examined specimens. The juvenile series may not be complete because the difference in number of limb pairs between stages is not constant. Juveniles were recovered with 8-11 pairs of trunk limbs, but two pairs of limbs and a bilobate flap are added between the last nauplius and earliest juvenile available here. Furthermore, no specimen with 8 pairs of setose trunk limbs was found, and the number of pairs of asetose limbs among the juveniles ranged from 0 to 2, although 1 pair of asetose limbs was most common. The posterior setose limb on specimen #26 could be considered a dimorphic expression of the first of two asetose limbs. If so, this specimen can be grouped among those with 5 setose and 2 asetose limb pairs, and a bilobate flap, but this dimorphic hypothesis does not explain specimen #12. In addition, according to Olesen (2005), the first juvenile instar of *L. brachyurus* has 6-8 pairs of setose trunk limbs, but it is unclear whether this means variation is truly present among juveniles of that stage, the precise number could not be established, or more than one juvenile instar was actually observed. The last naupliar stage of *L. biformis* and *L. brachyurus* has five pairs of asetose limbs.

Specimens were cleared and dissected in lactic acid, stained by adding a solution of chlorazol black E dissolved in 70% ethanol/30% de-ionized fresh water, and examined in glycerin with bright-field or differential interference optics. Drawings were made with a camera lucida.

Trunk limbs of *L. biformis*, like those of most branchiopods, are weakly sclerotized with arthrodial membranes unexpressed on the exopod and poorly expressed on the protopod and endopod. Nonetheless, protopodal and endopodal segments can often be identified by a broad, ventral projection or endite on the former and a lobe on each of the latter segments (Ferrari & Dahms, 2007). [N.B.: The protopodal structures called discoidal and attenuate lobes by Ferrari & Grygier (2003) are here re-named discoidal and attenuate endites.] Limb segment homologies follow from the limb patterning model of Ferrari & Grygier (2003) and consist of: a protopod with up to three segments — praecoxa, coxa and basis; a 3-segmented endopod; and an unsegmented exopod extending both proximally and distally. All of these bear setae. An exite, called an epipod by many authors, that arises ventral to the proximal extension of the exopod does not bear setae.

## RESULTS

Adult female. — On large adult females with 12 pairs of setose limbs and a bilobate flap, the praecoxal endite of limbs 1-7 is distinctly elongate ventrally and flexed proximally. Proximal to this endite is a thin, rod-like structure without setae (fig. 1). Both the coxal and the basal endites of limbs 1-7 are broad, and the lobe of endopodal segments 1-3 is elongate. The exopod has proximal and distal extensions, and the exite is shorter than the proximal lobe of the exopod. On limbs 8-12, the praecoxal endite is not flexed and is only slightly longer than the other endites (fig. 2A); the coxal and basal endites of limbs 8-12 are not broad, and are more similar in size and shape to the foreshortened lobes of endopodal segments 1-3. The distal extension of the exopod is progressively smaller on limbs 7-10 (fig. 2B), and absent on limbs 11-12; the proximal extension curves outward on limbs 8-10, and its setation is restricted to the tip of the extension on these limbs (fig. 2A). The proximal extension of the exopod is absent on limbs 11-12. An exite is absent on limbs 8-12.

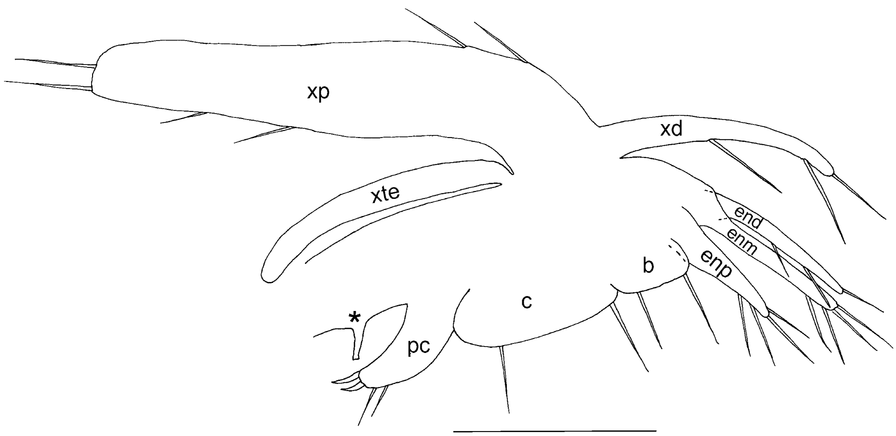


Fig. 1. *Lynceus biformis* (Ishikawa, 1895). Female. Setose trunk limb 1, proximal left, ventral down, scale line 1.0 mm. Abbreviations: b, endite of basis; c, endite of coxa; end, lobe of distal endopodal segment; enm, lobe of middle endopodal segment; enp, lobe of proximal endopodal segment; pc, endite of praecoxa; xd, distal extension of exopod; xp, proximal extension of exopod; xte, exite; asterisk next to rod-like structure.

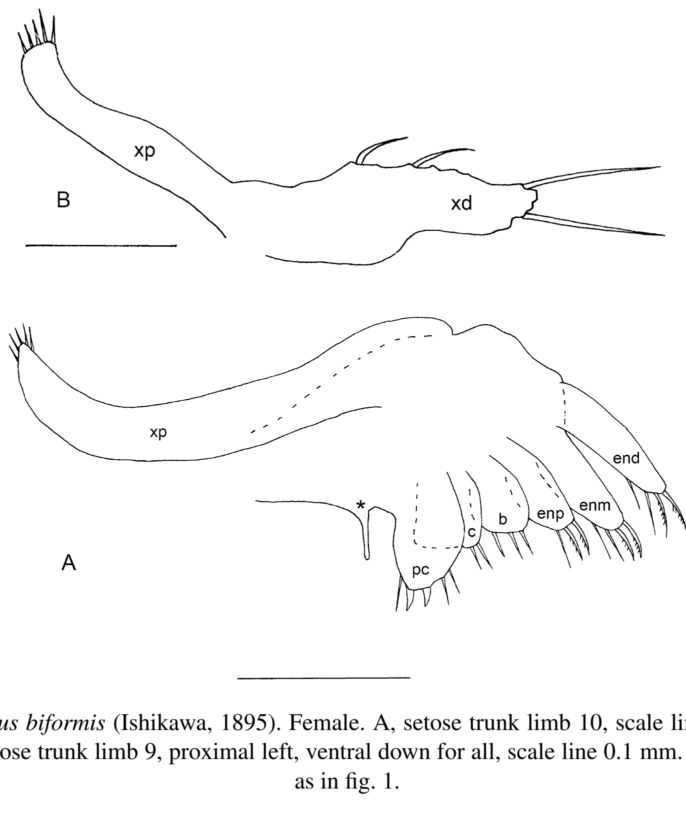


Fig. 2. *Lynceus biformis* (Ishikawa, 1895). Female. A, setose trunk limb 10, scale line 0.5 mm; B, exopod of setose trunk limb 9, proximal left, ventral down for all, scale line 0.1 mm. Abbreviations as in fig. 1.

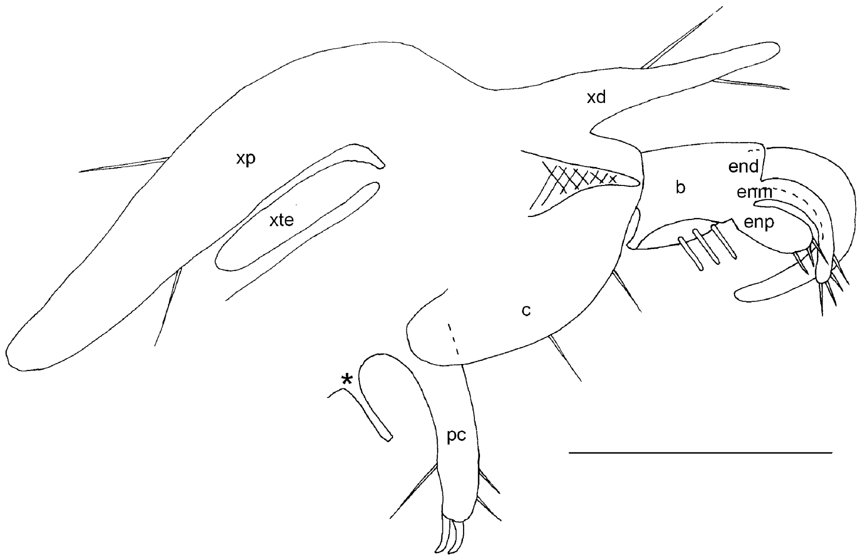


Fig. 3. *Lynceus biformis* (Ishikawa, 1895). Male. Setose trunk limb 1, proximal left, ventral down, scale line 1.0 mm. Abbreviations as in fig. 1.

Four females with 12 pairs of setose limbs plus a bilobate flap exhibit little variation among themselves in limb morphology despite a more than threefold difference in carapace size (length, 5.03 mm to 1.57 mm, and width, 4.47 mm to 1.33 mm). On the smallest female, that has 11 pairs of setose limbs plus a bilobate flap, trunk limb 11 has very few setae on the protopodal endites.

Adult male. — The number of protopodal endites and endopodal lobes, the rod-like structure, and the exopod and exite on limb 1 are identical to those of the adjacent limb. The distal segment of the endopod forms a subchela or "clasper" with the basis (fig. 3). Anterior-posterior changes in configuration of the remaining 9 pairs of setose limbs on adult males generally follow those of females with the exception of the exopod, the distal and proximal extensions of which both become progressively smaller on limbs 9-10 (fig. 4A, B).

Five males with 10 pairs of setose limbs plus a bilobate flap exhibit a more than 2.5 fold difference in carapace length (4.66 mm to 1.83 mm) and width (4.50 mm to 1.58 mm). There is little variation among these trunk limbs, except that the exopod on limb 10 appears as a small, undifferentiated mass dorsally on the two smallest specimens.

Juveniles. — On specimens with 11 limbs (either 10 pairs of setose limbs and a bilobate flap or 9 pairs of setose limbs, a pair of asetose limbs and a bilobate flap), the proximal and distal extensions of the exopod decrease in size on limbs 7-9, relative to their endopodal lobes. If limb 10 is setose, the exopod is reduced to

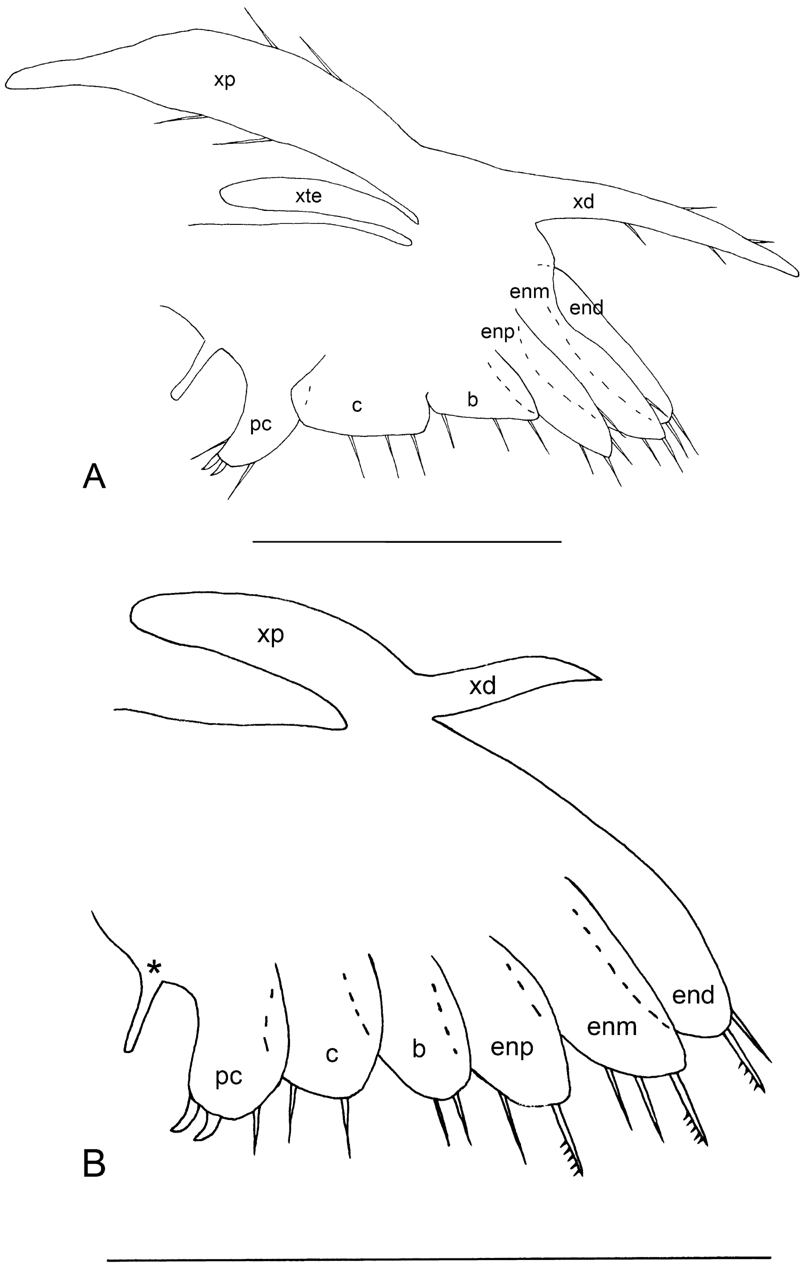


Fig. 4. *Lynceus biformis* (Ishikawa, 1895). Male. A, setose trunk limb 5, proximal left, ventral down, scale line 0.5 mm; B, setose trunk limb 10, proximal left, ventral down, scale line 0.5 mm. Abbreviations as in fig. 1.

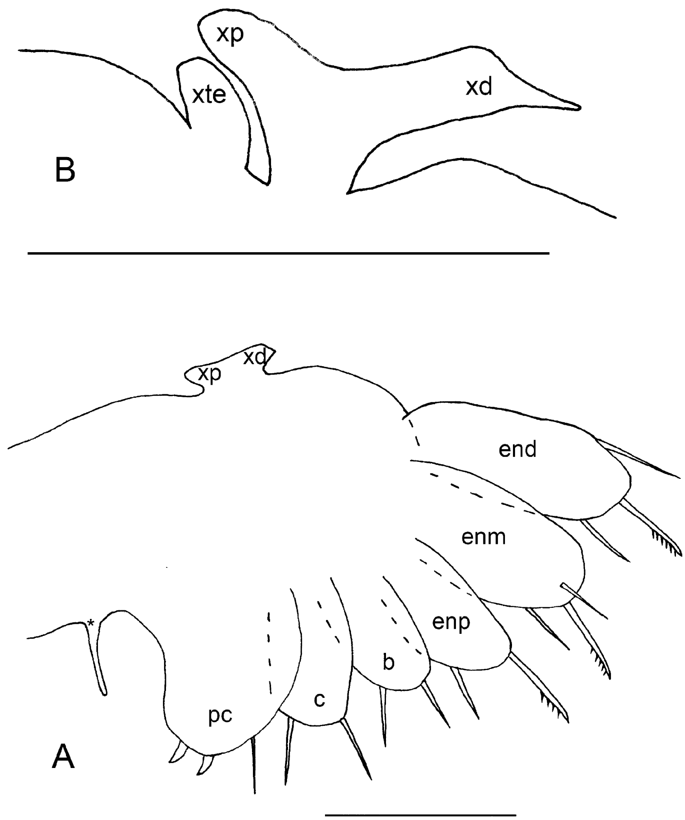


Fig. 5. *Lynceus biformis* (Ishikawa, 1895). Juvenile. A, setose trunk limb 9, proximal left, ventral down, scale line 0.1 mm; B, exopod and exite of setose trunk limb 7; pointed distal projection of exopod, proximal left, ventral down, scale line 0.1 mm. Abbreviations as in fig. 1.

a dorsal mass with very small proximal and distal extensions (fig. 5A). An exite is absent on limbs 8-10. Among different specimens, a proximal rod-like structure is absent on an asetose limb 10, but present on a setose one.

On juvenile specimens with 9 or 10 limbs (either 7 pairs of setose limbs, a pair of asetose limbs and a bilobate flap or 7 pairs of setose limbs, 2 pairs of asetose limbs and a bilobate flap), the praecoxal endite of limbs 1-3 is distinctly elongate ventrally and flexed proximally; on the remaining limbs, the praecoxal endite is not flexed and is only slightly longer than the other endites. The coxal and basal endites on limbs 1-3 are broad and partly fused; on the remaining limbs these endites are round and separate from each other. The exopod on limbs 1-7 has both proximal and distal extensions; an exite is present on all setose limbs (fig. 5B). On asetose limbs (limbs 8-9, or only limb 9), the exopod has a distal extension, and the exite and rod-like structure are absent (fig. 6).

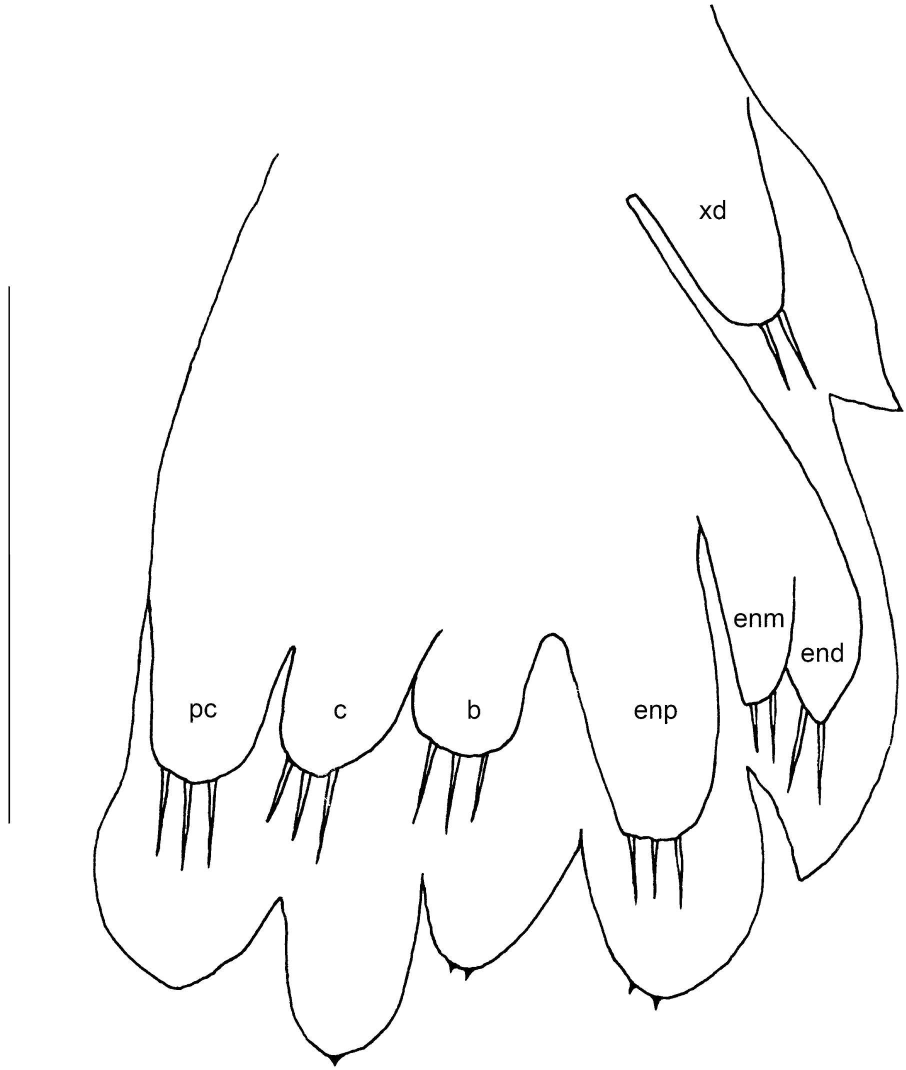


Fig. 6. *Lynceus biformis* (Ishikawa, 1895). Juvenile. Asetose trunk limb 7 and setose limb of following stage within, proximal left, ventral down, scale line 0.05 mm. Abbreviations as in fig. 1.

On the presumably earliest juvenile specimens with 5 pairs of setose limbs, 2 pairs of asetose limbs and a bilobate flap, the praecoxal endite of limbs 1-3 is distinctly elongate ventrally and flexed proximally; on the remaining limbs, the praecoxal endite is not flexed and is only slightly longer than the other endites. The coxal and basal endites of limbs 1-3 are partly fused but not broad; on the remaining limbs, the coxal and basal endites are rounded and distinct. The exopod of limbs 1-5 has small proximal and distal extensions, and an exite is present. On asetose limbs 6-7, there are altogether 6 endites and lobes, the exopod has a small distal extension, and there is neither an exite nor a rod-like structure.

Nauplii. — On a late nauplius with 5 pairs of asetose trunk limbs (the last naupliar instar of J. Olesen & M. J. Grygier, unpubl. data), all limbs are similar, with 4 distinct endites/lobes, but with no exopod, exite or rod-like structure. The proximal lobe, the one closest to the ventral surface of the body, is broader than the other lobes and on some limbs two developing lobes are present within its cuticle (fig. 7A).

## DISCUSSION

### Segmental homologies

Trunk limbs of *Lynceus biformis* are poorly sclerotized with no indication of arthrodial membranes separating segments. In contrast, weak arthrodial membranes have been reported on trunk limbs of spinicaudatans (Ferrari & Grygier, 2003), although these also have been interpreted as transverse folds not comparable to arthrodial membranes of other crustacean limbs (Olesen, 2007). The setose attenuate endite reported for spinicaudatans (Ferrari & Grygier, 2003) is absent on all trunk limbs of *L. biformis*, but a thin, rod-like structure without setae is found in a comparable position. On spinicaudatans, this attenuate endite (the middle praecoxal endite according to Ferrari & Grygier, 2003) is proximal to and closely associated with the distal praecoxal endite. In contrast, the rod-like structure of *L. biformis* arises separately and apart from the distal praecoxal endite. The rod-like structure is added after the distal praecoxal endite has been formed, as is true for the setose attenuate endite of spinicaudatans, but despite the positional and developmental identity it is not clear from morphology that the two structures are homologous.

The discoid endite of spinicaudatans (Ferrari & Grygier, 2003) is absent on all trunk limbs of *L. biformis*. On adult spinicaudatans, this setose endite is the proximal endite on the praecoxa of the trunk limbs. A similarly placed endite also has been observed on trunk limbs of *Eulimnadia texana* Packard, 1871 (cf. F. D. Ferrari, unpubl. data), and so this endite now is known from trunk limbs of representatives of all three extant spinicaudatan families: Limnadiidae, Leptestheriidae and Cyzicidae.

The transformed trunk limb 1, or "clasper", of males of *L. biformis* bears a well sclerotized subchela formed from the basis and the ventral lobe of the distal segment of the endopod; the proximal and middle endopodal segments are associated with this subchela. The "clasper" on males of spinicaudatans is

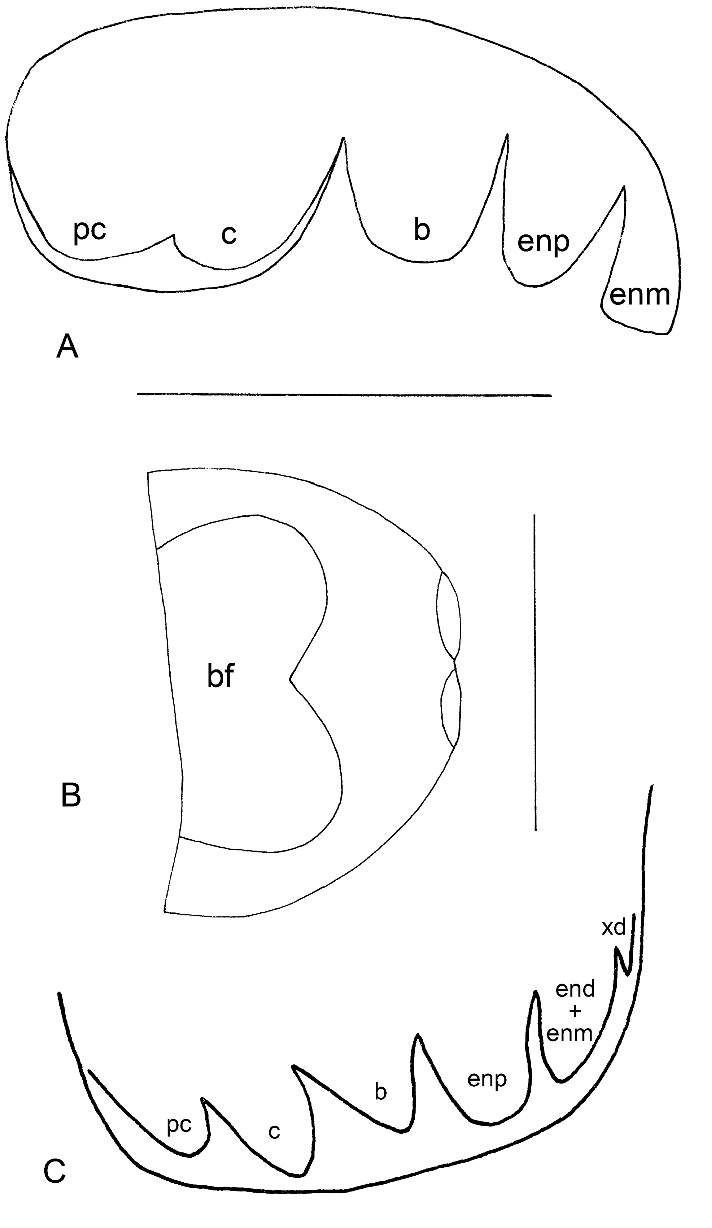


Fig. 7. *Lynceus biformis* (Ishikawa, 1895). Nauplius. A, asetose trunk limb 3, proximal left, ventral down, scale line 0.05 mm. Male. B, bilobate flap (bf) of trunk limb 10, anterior left, ventral down, scale line 0.1 mm. Juvenile. C, bilobate flap of trunk limb 8 and asetose limb of following stage within, proximal left, ventral down, scale line 0.05 mm. Abbreviations as in fig. 1.

a subchela formed differently, mainly from the distal endopodal segment and the proximal of two middle segments of the endopod, but with the distal of the two middle endopodal segments closely associated. This latter segment, the so-called "articulated palp", is composed of an elongate part representing the segment proper and an ordinary, unarticulated palp at its tip. There is nothing equivalent to this segment on the first or second female trunk limbs, and we interpret it as representing a positionally third but ontogenetically fourth endopodal segment (Ferrari & Grygier, 2003; Ferrari & Dahms, 2007). During spinicaudatan development, this unusual fourth endopodal segment is added to the limb during the transformation of the male trunk limb 1 into a "clasper". No such fourth endopodal segment develops on limb 1 of males of *L. biformis*.

A male limb that functions to clasp the female during copulation, and that is derived from inexactly corresponding subsets of homologous segments among different monophyletic lineages, initially was proposed to explain the structure of the male clasper of different 'conchostracan' groups of branchiopods (Olesen et al., 1996). Similar inexactly corresponding subsets of homologous segments are known to form another copulatory appendage, the chela-like fifth leg (sixth trunk limb) of males of some centropagoidean copepods (Ferrari & Ueda, 2005): a 'chela' is formed from the basis and distal segment of the exopod on males of species of *Temora*; from the proximal segment and a complex of the middle and distal segments of the exopod of males of species of *Pontella*; from the middle and distal segments of the exopod of males of species of *Centropages*.

A 3-segmented interpretation of the endopod in non-clasper-like trunk limbs has been proposed for the spincaudatans *Caenestheriella gifuensis* and *Leptestheria kawachiensis* by Ferrari & Grygier (2003), *Limnadopsis parvispinus* (Henry, 1924) by Pabst & Scholtz (2009), and for extant branchiopods by Boxshall (1998). An alternative hypothesis is that the endopod is unsegmented on all extant branchiopods (Olesen et al., 1996; Olesen, 2007). The Upper Cambrian branchiopod *Rehbachiella kinnekullensis* Müller, 1983 has a 4-segmented endopod (Walossek, 1993). Several interpretations of the protopod also have been proposed: a tri-enditic praecoxa, a coxa and a basis on *C. gifuensis* and *Leptestheria kawachiensis* (see Ferrari & Grygier, 2003); only a coxa and a basis on *Limnadopsis parvispinus* (see Pabst & Scholtz, 2009) and on all extant branchiopods (Boxshall, 1998); or an undifferentiated protopod (Walossek, 1993; Olesen, 2007). In support of their hypotheses of segment homologies of the protopod and endopod, Ferrari & Grygier (2003) provided a model of limb patterning during development of spinicaudatans.

## Trunk limb development

The simplest and smallest trunk limb pair is the posterior bilobate flap, found on all post-naupliar specimens. These flaps were described as "opercular lamellae" on *L. biformis* by Yoon & Kim (2000), as well as on *L. gracilicornis* by Martin et al. (1986), although only the latter authors considered the lamella to represent a limb pair. There is never more than one bilobate flap present, and on post-naupliar specimens it is the posterior limb. There is no indication of protopodal endites, endopodal lobes, an exopod or an exite on this simple structure (fig. 7B). Because no such flap representing the sixth pair of limbs is present on late nauplii with five pairs of asetose trunk limbs, it is clear that not all limbs begin development as a bilobate flap, but it is not clear which sequential pair is the first to do so.

For posterior limbs, though, clear evidence exists of the flap's role as a stage in limb development. On one juvenile (#26) with 6 pairs of setose limbs, 1 pair of asetose limbs and a bilobate flap, a bilaterally paired structure with six projections appears within the cuticle of the bilobate flap; this is the developing step of the eighth limb pair (fig. 7C). The distal projection, towards the dorsal axis of the body, is much thinner than the other five projections and does not extend as far ventrally as the others. We interpret the six projections as the precursors of the praecoxal, coxal and basal endites, the proximal and middle endopodal segments, and the exopod.

Within the cuticle of asetose limb 7 of the same specimen, a setose limb 7 is developing (fig. 6) with seven setose, ventral projections including six large ones and a smaller, thinner, distal one that does not extend as far ventrally. Two setose projections appear within the penultimate ventral projection of the asetose limb. On asetose limb 7, we interpret the six projections as the precursors of the praecoxal, coxal and basal endites, the lobes of the proximal and middle endopodal segments, and the exopod. On the developing setose limb 7, the seven projections are clearly precursors of the praecoxal, coxal and basal endites, the lobes of the proximal, middle and distal endopodal segments, the distal endopodal segment being newly formed, and the exopod.

The situation for more anterior limbs appears to differ. Asetose limbs 1-5 of the late nauplius, that are unaccompanied by a bilobate flap, have four ventral projections each (fig. 7A). On juveniles, presumably representing the next stage in development, with 5 pairs of setose limbs, 2 pairs of asetose limbs and a bilobate flap, the setose limbs each have seven projections, that we interpret as the praecoxal, coxal and basal endites, the lobes of the proximal, middle and distal endopodal segments, and the exopod. We interpret the four projections on each asetose naupliar limb as a syncoxal endite, the basal endite, and the lobes of the proximal and middle segments of the endopod. The two structures within the cuticle of the broad syncoxal endite presumably will appear after the next molt as separate and distinct endites of the praecoxa and coxa, and the distal endopodal segment will be the last segment added to the ramus.

An exite and rod-like structure are absent on naupliar asetose limbs 1-5. They are present on setose limbs 1-5 of juveniles with 5 pairs of setose limbs, 2 pairs of asetose limbs and a bilobate flap but are not on asetose limbs 6-7 of this stage. They also are absent on asetose limbs 8-9 of juveniles with 7 pairs of setose limbs, 2 pairs of asetose limbs and a bilobate flap. Thus, the exite and the rod-like structure initially appear during the molt from an asetose to a setose limb.

On early juvenile stages of *L. biformis*, the setose anterior limbs appear more developed than are asetose posterior limbs of later juvenile stages. The anterior limbs have a complete set of protopodal endites and endopodal lobes from the start of the juvenile phase, whereas at least the distal endopodal segment is missing on the asetose posterior limbs and only develops in the earliest setose limb stage. This condition is to some degree similar to that of spinicaudatans (Ferrari & Grygier, 2003), in which limb 6 was the standard of comparison. In *Leptestheria kawachiensis*, this limb was followed from the asetose limb stage to the adult limb: all praecoxal and endopodal lobes are present from the start in limb 6, but the distal endopodal lobe is absent in the asetose stage of at least limb 13 and more posterior limbs. The comparison is imperfect, however, since it was not possible to compare anterior and posterior asetose limbs of juveniles of *Lynceus biformis*. The first five pairs, which were asetose limbs in the late nauplius, were all setose limbs in the earliest available juveniles, unlike in *Leptestheria kawachiensis*. Nonetheless, it is possible to state that an exite and a rod-like structure are later additions to the laevicaudatan limb, just as the exite, setose attenuate endite and discoid endite are on spinicaudatans.

In contrast, the naupliar asetose limbs 1-5 of the laevicaudatan begin development with fewer endites and lobes (four) than the more posterior asetose limbs of juveniles (six). The same is true to an even greater degree in *L. brachyurus* (cf. Olesen, 2005), in which the anterior naupliar trunk limbs have "about five" lobes or "limb parts", but more posterior naupliar limbs appear to have only two. Olesen (2005) stated that the lower number of limb parts in a naupliar limb than in the corresponding adult limb means that no complete homology scheme can be established; nonetheless, based on our earlier spinicaudatan results, we have proposed such a scheme here (fig. 7A and above) for the naupliar trunk limbs of *L. biformis*. Among the similarly configured swimming legs of copepods, anterior limbs of late nauplii are more developed than posterior limbs of juveniles (Ferrari & Dahms, 2007).

Comparisons of limb number among late nauplii of laevicaudatans and spinicaudatans also is informative. The late nauplii of both *Lynceus biformis* (examined herein) and *L. brachyurus* (cf. Olesen, 2005) have five pairs of asetose trunk limbs, thereby differing in two respects from the last naupliar stages of spinicaudatans *Caenestheriella gifuensis* and *Leptestheria kawachiensis*, i.e., in the number of limb pairs (five versus seven or eight) and in the absence of transverse limb stripes on the laevicaudatans. Transverse limb stripes are an apparently very early step in the development of branchiopod limbs. The last naupliar stage of *Caenestheriella gifuensis* has eight pairs of limbs, expressed as four pairs anteriorly with setae short and not articulating at their bases, followed by one asetose pair, and three pairs of transverse limb stripes (Olesen & Grygier, 2004). The eight or nine pairs of trunk limbs of the last naupliar stage of *Leptestheria kawachiensis* are similar, but with the first five pairs with unarticulating setae, two asetose limbs, and one or two limb stripes (Ferrari & Grygier, 2003; J. Olesen & M. J. Grygier, unpubl. SEM images). Naupliar trunk limbs of *Eulimnadia braueriana* Ishikawa, 1895 are again similar: four pairs with unarticulating setae, one asetose pair, and three pairs of transverse limb stripes (Olesen & Grygier, 2003). In the Methods section herein, we regarded the addition of two limb pairs and a bilobed flap between the late nauplius and the youngest available juvenile of *Lynceus biformis* as a possible indication of a missing stage. Actually, there is probably little cause for concern. The youngest juveniles of *Leptestheria kawachiensis* available to Ferrari & Grygier (2003) had 13 pairs of trunk limbs at different stages of development, representing an even greater increase in leg pair number compared to the late nauplius of that species.

### Anterior/posterior pattern

Two kinds of changes are evident among trunk limbs along the anterior/posterior axis. Sexually dimorphic structures on adults appear to result from sudden changes for which no transitory state is evident among juveniles. These include a clasper on trunk limb 1 of males, and on females the exopod of trunk limbs 8-12 with a curved proximal extension and a weakly developed distal extension (limbs 8-10) or no distal extension (limbs 11-12). The male clasper and a female exopod with a curved proximal extension initially were described by Martin et al. (1986) for *L. gracilicornis*.

The second kind of change includes those that begin on juveniles. They include changes on limbs 4-7, which in juveniles initially have: a praecoxal endite only slightly longer than the other endites and that is not flexed; a coxal and a basal endite that are rounded and distinct; and lobes of endopods 1-3 that are similar in size to the endites. Later in development, the praecoxal endite of limbs 4-7 is distinctly elongate ventrally and flexed proximally, the coxal and basal endites are broad and partly fused, and the lobes of endopods 1-3 are elongate. Trunk limbs 1-3 do not pass through a step in which the endites and lobes are similar in size, while trunk limbs 8-12 retain that condition throughout their development.

TABLE II

Occurrence of selected structures on trunk limbs 1-12 (1-10 in ♂) of adult *Lynceus biformis* (Ishikawa, 1895)

| Structure and states | Trunk limbs |
|---|---|
| Praecoxal endite elongate, flexed proximally/short and unflexed | 1-7/8-12 |
| Endites of coxa and basis broad/narrow | 1-7/8-12 |
| Endopodal lobes long/short | 1-3/4-12 |
| Exite present/absent | 1-7/8-12 |
| (♀) Distal extension of exopod normal/shortened/absent | 1-6/7-10/11-12 |
| (♀) Proximal extension of exopod normal/ bent outward with only distal setation/absent | 1-7/8-10/11-12 |
| (♂) Exopod normal/proximal and distal extensions both reduced | 1-8/9-10 |

As part of their analysis of spinicaudatan trunk limb structure, Ferrari & Grygier (2003) tabularized the anterior-to-posterior occurrence of selected structures on the trunk limbs of female *Caenestheriella gifuensis*. Here we have done the same for *L. biformis* (table II). In both species, the praecoxal endite is flexed proximally only in the anterior two-thirds of the limb series, but fewer features show restricted ranges or drop out posteriorly in the present laevicaudatan than in the spinicaudatan. This is partly due to the total absence in *L. biformis* of some structures, such as the discoid endite and the palp on enditic lobe 5 (= endopodal segment 2), but on *L. biformis* the praecoxal rod-like structure is present on all limbs, while its possible homologue, the attenuate endite, is missing from the last six pairs of *C. gifuensis*. Conversely, the exite is missing from several posterior limbs in *L. biformis*, but not *C. gifuensis*. The gradual and sexually dimorphic reduction of the exopodal extensions in *L. biformis* posterior to limb 7 or 8, and the absence of the exopod in the most posterior limbs of the female, are different in detail from the pattern observed in *C. gifuensis*, in which, for example, even the last limb has a setose exopod. Ferrari & Grygier (2003) concluded that abrupt changes in limb structure along the anterior-posterior axis might be traces of an ancestral trunk tagmosis in the Spinicaudata. Such abrupt changes are not prominent in *L. biformis*, however, and provide scant evidence of residual trunk tagmosis in the Laevicaudata. The point of greatest change lies perhaps between limbs 7 and 8, but the posterior limbs are perhaps better characterized as being less fully developed than as forming a separate functional unit.

## ACKNOWLEDGEMENTS

This report is a product of Cooperative Research Project K00-07 of the Lake Biwa Museum, "Studies on Large Branchiopods (Fairy Shrimp, Tadpole Shrimp, and Clam Shrimp) Inhabiting Rice Paddies". We extend special thanks to Lana Ong (National Museum of Natural History, Smithsonian Institution) for specimen and figure preparations.

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First received 17 November 2011.
Final version accepted 30 December 2011.