

MORPHOLOGY AND SYSTEMATIC SIGNIFICANCE OF THE MYSTAX,  
A HITHERTO UNDESCRIBED CEPHALIC STRUCTURE OF MALES  
IN CERTAIN NOTOSTRACA (BRANCHIOPODA)

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A B S T R A C T

We report on the morphology and occurrence of the “mystax,” an overlooked cephalic structure found in males of certain Notostraca. The mystax is a conspicuous, protuberant, dark-brownish sclerodermal stripe located on the anteroventral flange of the carapace. Histological sections show the mystax as an expansion of the exoskeleton formed by the exocuticle. Examination of exuviae revealed that the old mystax remains intact in the shed cuticle. In some *Triops* populations of México and Japan, large individuals bearing ovisacs exhibit a “pseudomystax,” which consists of a dark-brownish band in nearly the same position as the mystax, but it does not form a protuberant stripe. A total of 661 males and 6402 ovisac-bearing individuals from four continents were examined. The mystax was found in males of *Triops australiensis* from Australia, *T. granarius* from Japan, *Triops* sp. from North America, *Triops* sp. from Africa, and *Triops* sp. from Asia. The mystax is absent in all ovisac-bearing individuals of all species analyzed and in all males of *Triops cancriformis* (Africa, Europe, and Japan), and in all males of the genus *Lepidurus*. Practical and systematic implications of the mystax are discussed.

INTRODUCTION

The presence of a hemispherical ovisac in each of the eleventh thoracopods of the adult tadpole shrimp (the endopod and exopod of these appendages being modified to form an oöstegopod) is the character that unequivocally separates females and hermaphrodites from males. In addition, the most common sexually dimorphic features reported within the Notostraca are: 1) the second pair of thoracopods has the fifth endites commonly longer than the sixth endites in males, while in ovisac-bearing individuals they are more nearly equal (Longhurst, 1955); 2) the number of body rings without thoracopods is greater in males than in ovisac-bearing individuals; thus, for a similar body size, the number of body rings with thoracopods is greater in ovisac-bearing individuals than that in males (Sars, 1899; Barnard, 1924; Uéno, 1940; Longhurst, 1955); 3) the male carapace is typically broader laterally but shorter longitudinally than that in ovisac-bearing individuals (less marked in *Lepidurus* than in *Triops*), with considerable overlap between individuals (Packard, 1874; Uéno, 1940; Longhurst, 1955; Saunders, 1980); 4) the maxillary efferent ducts are longer in males than in ovisac-bearing individuals in *Triops* (Longhurst, 1955); 5) the spines on the ventral side of the proximal region of the cercopods tend to be broader in males than in ovisac-bearing individuals, often forming protuberant scales in *Triops* (Gurney, 1924; Longhurst, 1955).

During a study on Mexican notostracans, one of us (HOB) found that males of the genus *Triops* bore a distinctive

structure on the anterior-ventral side of the carapace that was absent in all ovisac-bearing individuals. She further looked for this structure, here named the mystax (Latin for moustache), in males and ovisac-bearing individuals of *Lepidurus lemmoni* Holmes, 1894 from México and *Triops cancriformis* (Bosc, 1801) from Europe, and found that it was absent in all specimens analyzed. Using material from Africa, Asia, Australia, Europe, and North America, we carried out a study on the presence or absence of the mystax in the Notostraca. The objectives of this work are: 1) to provide a morphological description of the mystax, 2) to analyze its occurrence across the order, 3) to discuss its relevance to the systematics of the group, and 4) to demonstrate the practical significance of the mystax.

MATERIALS AND METHODS

The material analyzed came from the Australian Museum (Sydney) (AM), Victoria Museum (Melbourne, Australia) (VM), Lake Biwa Museum (Japan) (LBM) [including some specimens borrowed from the Kurashiki Museum of Natural History (KMNH)], Centro de Investigaciones Biológicas del Noroeste, S.C. (México) (CIBNOR), Universidad Autónoma de Nuevo León (México) (FCB, UANL), Universidad Juárez del Estado de Durango (México) (UJED), and the collections of D. Christopher Rogers (DCR). Field samples obtained from Africa, Mongolia, and México deposited in the CIBNOR collection were also included. Animals were examined under a stereoscopic microscope and sexed based on the presence or absence of the above-mentioned ovisacs, and the presence or absence of the mystax was registered. The absence or presence of the mystax during the postlarval development of *Triops* sp. was followed in cultured animals

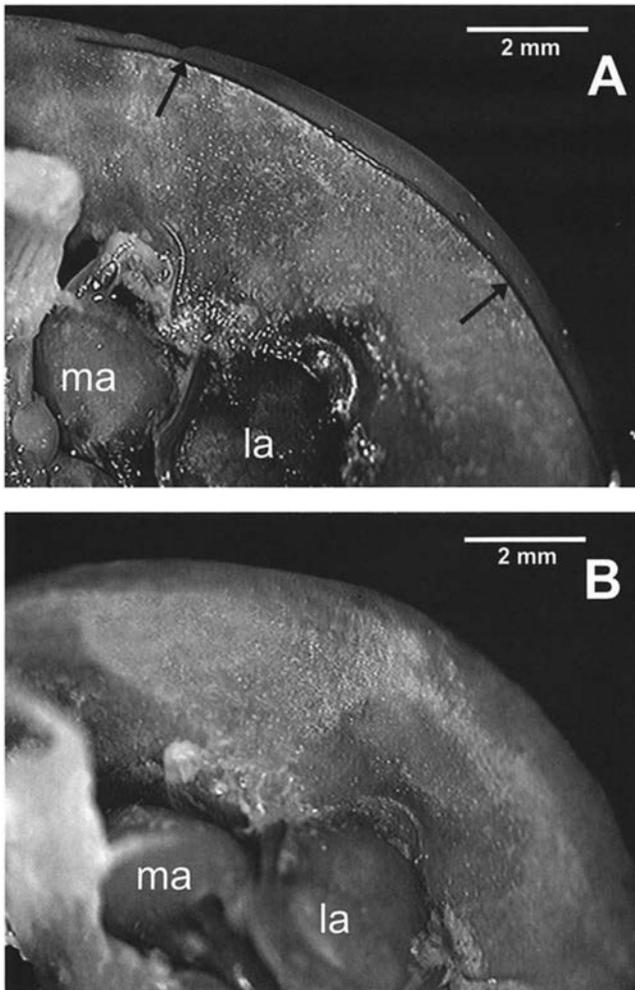


Fig. 1. A-B: Anteroventral flange of carapace of *Triops* sp. from Zacatecas, México. A, Male; the mystax is indicated by the arrows. B, Ovisac-bearing individual. la, labrum; ma, mandible.

from Baja California (Norte), Baja California Sur, and Sonora, México. Specimens were cultured outdoors using potable water and soil from the habitat as substrate and as a source of cysts. Males and ovisac-bearing individuals of *Triops* sp. from Zacatecas, México, and ovisac-bearing individuals of *Triops* sp. from Baja California (Norte), were fixed in Davidson's solution (24 h) and dehydrated in 70, 80, 90, and 100% ethanol, and paraffin-embedded serial sections (6  $\mu$ m) were stained with hematoxylin and eosin (Bell and Lightner, 1988) for structural examination of the mystax under a light microscope.

## RESULTS

### Morphological Description of the Mystax

The mystax is a conspicuous, protuberant, dark-brownish sclerodermal stripe located on the anteroventral flange of the carapace far anteriorly on the ventral side of the cephalon. It extends for about 80% of the curved anterior border (Fig. 1A), but its extent, and the span of its colored portion, vary somewhat among specimens. Histological sections of *Triops* sp. (Zacatecas, México) show the mystax as an expansion of the exoskeleton formed by the exocuticle (Fig. 2A). It takes the form of a distinct ridge. Examination of exuviae of

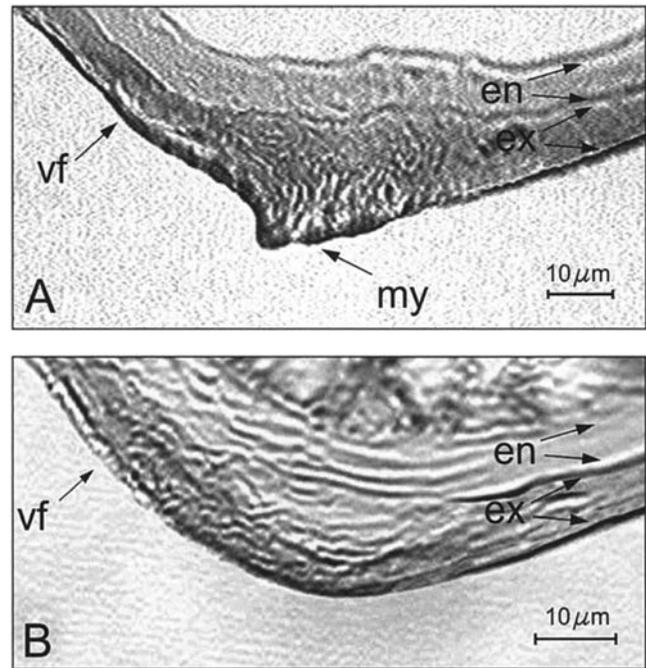


Fig. 2. A-B. Sagittal section of the anterior part of the carapace. A, Male of *Triops* sp. from Zacatecas, México. B, Ovisac-bearing individual from Baja California (Norte), México. en, endocuticle; ex, exocuticle; my, mystax; vf, anteroventral flange of carapace.

specimens of the same population revealed that after molting the old mystax remains intact in the shed cuticle. The molting suture along the front of the exuviae is located at the anterior border of the carapace just in front of the mystax (Fig. 3A, B). In newly molted males of Japanese *T. granarius* (Lucas, 1886) preserved in ethanol the mystax was uncolored. In reared *Triops*, while the ovisacs were evident in immature individuals of at least 6.0 mm in carapace length, the mystax was visible in immature males of at least 7.9 mm in carapace length.

In *Triops* populations of México (CIB-546 and 533) and Japan (CIB-717), some extremely large individuals bearing ovisacs have a dark-brownish band in nearly the same position as the mystax, but it does not form a protuberant ridge and merely marks the lower boundary of the thick cuticle of the dorsal shield where it meets the distinctly thinner ventral cephalic cuticle. We name this feature the "pseudomystax" (Fig. 3C, D).

### Occurrence of the Mystax within the Notostraca

In total, 661 males and 6402 ovisac-bearing individuals (females and/or hermaphrodites) were examined (Table 1). The mystax was found in all males of *Triops australiensis* (Spencer and Hall, 1896) from Australia, *T. granarius* (Lucas, 1864) from Japan, *Triops* sp. from North America, *Triops* sp. from Africa, and *Triops* sp. from Asia. The mystax is absent in all ovisac-bearing individuals of all species analyzed and in all males of *Triops cancriformis* (Africa, Europe, and Japan), and in all males of the genus *Lepidurus*.

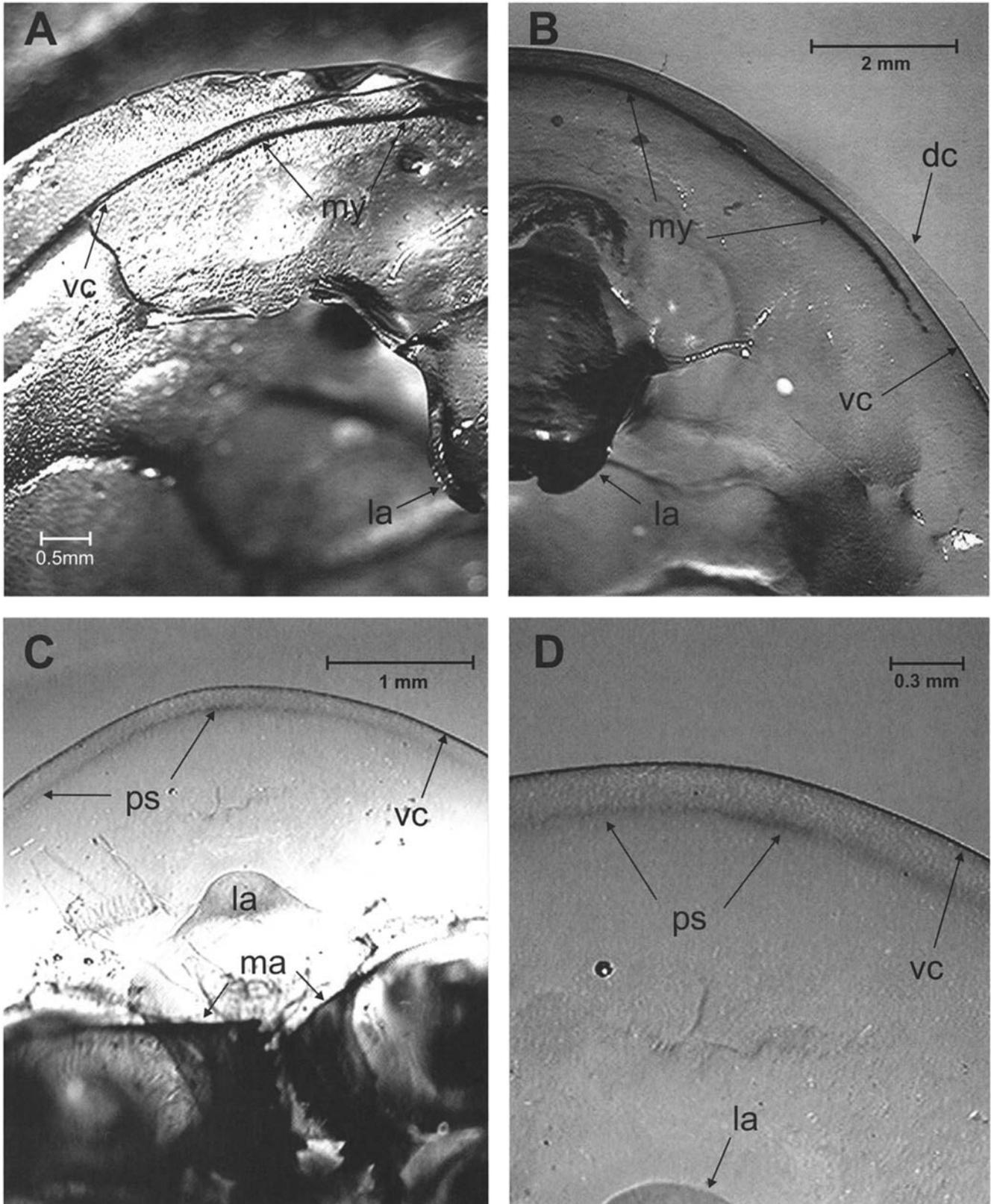


Fig. 3. A-B. Exuvium of male *Triops* sp. from Aguascalientes, México; C-D. Exuvium of ovisac-bearing individual of *Triops* sp. from Baja California (Norte), México. A, Right ventral side of carapace. B, Left ventral side of carapace; the dorsal (dc) and ventral (vc) borders of the molting suture are separated. C, Anteroventral flange of carapace. D, Detail from C. dc, dorsal border of molting suture; la, labrum; ma, mandible; my, mystax; ps, pseudomystax; vc, ventral border of molting suture.

Table 1. Presence of a mystax in the notostracan forms analyzed.

Species	Country	N	Males with mystax	Males without mystax	Ovisac-bearing individuals without mystax	Year of field collection
<i>Lepidurus apus</i> (Linnaeus, 1758)	Mongolia	5	—	4	1	—
<i>L. articus</i> (Pallas, 1793)	U.S.A.	5	—	—	5	—
<i>L. bilobatus</i> Packard, 1883	U.S.A.	10	—	5	5	1998-2000
<i>L. couesii</i> Packard, 1875	Canada	4	—	2	2	1998
<i>L. couesii</i>	U.S.A.	1	—	—	1	1956
<i>L. cryptus</i> Rogers, 2001	U.S.A.	53	—	—	53	1989-1999
<i>L. lemmoni</i> Holmes, 1894	México	34	—	22	12	2003
<i>L. lemmoni</i>	U.S.A.	57	—	28	29	1862-1999
<i>L. packardii</i> Simon, 1886	U.S.A.	19	—	—	19	1983-1993
<i>L. sp.</i>	France	5	—	3	2	—
<i>L. sp.</i>	Mongolia	1	—	—	1	—
<i>Triops australiensis</i> (Spencer and Hall, 1896)	Australia	228	81	—	147	1868-2004
<i>T. cancriformis</i> (Bosc, 1801)	France	16	—	1	15	1957-2001
<i>T. cancriformis</i>	Italy	3	—	—	3	1976
<i>T. cancriformis</i>	Japan	38	—	—	38	2001-2003
<i>T. cancriformis</i>	Malta	5	—	—	5	1977-1989
<i>T. cancriformis</i>	Morocco	7	—	3	4	2000-2001
<i>T. cancriformis</i>	Portugal	10	—	3	7	2001
<i>T. cancriformis</i>	Senegal	8	—	—	8	1991
<i>T. cancriformis</i>	Spain	10	—	5	5	2001
<i>T. cancriformis</i>	Turkey	2	—	1	1	1988
<i>T. cancriformis</i>	Yemen	2	—	—	2	—
<i>T. granarius</i> (Lucas, 1864)	Japan	305	199	—	96	1962-2001
<i>T. longicaudatus</i> (LeConte, 1846)	Japan	300	—	—	300	1976-2001
<i>Triops sp.</i>	Jordan	20	12	—	8	1978
<i>Triops sp.</i>	Lesotho	9	7	—	2	1998
<i>Triops sp.</i>	Mauritania	90	29	—	61	1977
<i>Triops sp.</i>	México	5696	225	—	5436	1980-2004
<i>Triops sp.</i>	Mongolia	29	10	—	19	2002
<i>Triops sp.</i>	South Africa	21	12	—	9	1992-1994
<i>Triops sp.</i>	U.S.A.	68	8	—	60	1995-2003
<i>Triops sp.</i>	Zimbabwe	2	1	—	1	1992

## DISCUSSION

## Systematics

The validity of *Lepidurus* and its systematic position with respect to *T. cancriformis* has been a moot point for a long time. According to Baird (1852), Milne Edwards, in his publication of 1840, did not accept the genus *Lepidurus* proposed by Leach in 1816 on the basis of the supra-anal plate, but only the genus *Triops* (cited as *Apus*). However, Baird (1852) accepted both genera and, since his publication, these taxa have been widely accepted by carcinologists (see Linder, 1952; Longhurst, 1955; Fryer, 1988), with the exception of Braem (1893). Ghigi (1921) proposed a further division of the Notostraca, distinguishing a genus *Proterothriops*, based on the shape of the carapace and the nuchal organ. Gurney (1924) and Barnard (1929) soon rejected Ghigi's proposal, demonstrating that the supposedly diagnostic characters were not of generic importance. Linder (1952) mentioned the close morphological resemblances between *Lepidurus* and *T. cancriformis* in telson structure, presence of second maxillae, and total number of body rings. The absence of the mystax should now be added to this list.

The separation of *Lepidurus* and *T. cancriformis* from those species of *Triops* in which the mystax is present, is not consistent with the presence (or absence) of second maxillae in the adult stage. Longhurst (1955) used this character as

one of the most important features to separate his four recognized species of *Triops* (*T. cancriformis* and *T. granarius* with, and *T. australiensis* and *T. longicaudatus* (LeConte, 1846) without second maxillae). If a character state polarization is proposed based on ontogenetic criteria (Nelson, 1978; Kitching, 1992), the presence of the second maxillae in adult specimens is scored as the plesiomorphic state of the character because these appendages are present in the metanauplius stage (Longhurst, 1955; Fryer, 1988). Also, by any sort of out-group comparison, presence of second maxillae is plesiomorphic. Thus this feature cannot be cited in support of a sister group relationship between *T. cancriformis* and *T. granarius*. Moreover, inconsistencies in the expression of the second maxillae in Old World species have been found. Longhurst (1958) reported an irregular and often asymmetrical reduction of the second maxillae in some samples of *T. cancriformis* and *T. granarius*, and even the complete absence of it in African and Asian specimens of the latter species. A case similar to that of the second maxillae occurs in the second antennae. The absence or presence of this appendage in Spanish material was used by Alonso (1996) as an additional character to separate specimens of the genus *Lepidurus* from specimens of the genus *Triops*. However, Longhurst (1955) mentioned that this appendage, although present in the larval stage, is often absent in large specimens of all species. In

agreement with Longhurst's statement, we found that the second antenna exhibits much the same range of irregular and asymmetrical reductions as the second maxillae in specimens of both genera.

Molecular phylogenetic analyses support the monophyly of the mystax-bearing forms (see Murugan et al., 2002, Figs. 5 and 7). Comparing sequences of the mitochondrial 12S rRNA gene of *Triops* spp. (North America), *T. australiensis* (Australia), *T. cancriformis* (Austria), and *T. granarius* (Japan), Murugan et al. (2002) reported the lowest Kimura 2-parameter distances between *T. australiensis* and the North American *Triops* forms (0.119-0.124), and between *T. granarius* and the North American *Triops* forms (0.138-0.148). Also, comparing sequences of the same mitochondrial gene and of the nuclear EF1 $\alpha$  gene, Braband et al. (2002) reported a closer relationship of *T. australiensis* and the North American *T. longicaudatus* than between these other species and *T. cancriformis*. More recently, Mantovani et al. (2004) concluded that *T. longicaudatus* is possibly more closely related to *T. granarius* (based on 16S rRNA sequences) and *T. australiensis* (by 12S sequences) than to *T. cancriformis*.

Despite the close morphological resemblance between *Lepidurus* and *T. cancriformis* mentioned above, molecular data suggest that *Lepidurus* forms a monophyletic group (Montovani et al., 2004) apart from the *T. cancriformis* group. Murugan et al. (2002) reported a higher Kimura 2-parameter distance value (based on 12S sequences) between *Lepidurus* and *T. cancriformis* than between *Lepidurus* and *T. longicaudatus*. Mantovani et al. (2004) showed that some molecular analyses clustered non-*T. cancriformis* forms with *Lepidurus*, and even the last entity did not show clear genetic differences at the genus level. Thus, in the systematics of the Triopsidae at least two questions remain to be solved: 1) Are *Lepidurus* and *Triops* monophyletic or not? 2) If one group is not monophyletic, what are the phylogenetic relationships of the different taxa?

#### Practical Significance

In Japan, the presence of three species of *Triops*, supposedly all introduced, has been demonstrated morphologically (Akita, 1976). They are called *T. longicaudatus*, *T. granarius*, and *T. cancriformis* there, but which overseas forms they precisely correspond to has not yet been determined. Of the three, only *T. granarius* has males in Japan, and no population of this species there lacks males: the other two species are evidently hermaphroditic, if not parthenogenetic. Within Japan the ranges of *T. longicaudatus* and *T. granarius* extensively overlap, e.g., Iguchi (2003), and close co-occurrence is not unusual. Distinguishing the two forms is firstly done based on the dorsal spines of the telson (Longhurst, 1955), but this has proven difficult for the lay volunteers who have collected much of the material for distribution surveys at the Lake Biwa Museum (Grygier et al., 2002). If specimens with a mystax are present in a Japanese sample, it is instantly clear that they are males of *T. granarius*. Also, unlike telson spines, the relatively large and darkly pigmented mystax may be a useful field character. Finally, when sexing individuals in a sample, it is much faster to isolate the males using the

presence or absence of the mystax, a feature that is instantly visible (cf. Fig. 1), than to search among the thoracopods for ovisacs.

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