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FIRST RECORD OF *LIMNADIA LENTICULARIS* MALES IN EUROPE (BRANCHIOPODA: CONCHOSTRACA)

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A B S T R A C T

Limnadia lenticularis, a well-known representative of the Conchostraca Spinicaudata in Europe, was generally accepted as a parthenogenetic species. During an intensive faunistic study of Austrian conchostracans, four of a total of 364 collected *L. lenticularis* specimens were males. This paper provides the collection localities, compares the sex ratios of the five conchostracan species found in Austria, and describes the general morphology of *L. lenticularis* males, focussing on SEM of the male claspers.

Limnadia lenticularis (Linné, 1761) is a well-known representative of the Conchostraca Spinicaudata. That it is an entirely parthenogenetic species, because no males were ever recorded in Europe, was generally accepted (Sars, 1896; Gislén, 1937; Mathias, 1937; Gruner, 1993; Schminke, 1996; Defaye *et al.*, 1998). However, two males were found recently in Florida by J. W. Martin (Sassaman, 1995).

The conchostracan fauna of the Austrian Morava and Danube river wetlands was summarized by Vornatscher (1968) and Löffler (1993) and recently has been re-investigated as part of a faunistic project on large branchiopods in Austria (Hödl and Eder, 1996a, b; Eder *et al.*, 1997). Today the Austrian distribution of *L. lenticularis* is limited to this region (Fig. 1). In addition to *L. lenticularis,* another species of the Limnadiidae, *Imnadia yeyetta* Hertzog, 1935, two representatives of the Leptestheriidae, *Eoleptestheria ticinensis* (Balsamo-Crivelli, 1859) and *Leptestheria dahalacensis* (Rüppell, 1837), and one species of the Cyzicidae, *Cyzicus tetracerus* (Krynicki, 1830), were documented in the Morava and Danube River wetlands. In 1997, while sampling for a molecular phylogenetic analysis of branchiopods (Richter *et al.*, in prep.), we found one *L. lenticularis* specimen with two pairs of claspers. A re-investigation of all conchostracans collected between 1994 and 1997 in Austria revealed that of a total of 364 *L. lenticularis* specimens, four were males.

MATERIALS AND METHODS

The *L. lenticularis* males were deposited with the Naturhistorisches Museum Wien (Crustacea Coll. Nr. NHMW 18394). For morphological comparison, males of the second species of Limnadiidae in central Europe, *I. yeyetta*, were sampled at the locality of males 1 and 2 on 23 June 1995.

Collected specimens were stored in 70% ethanol. In 1998, two *L. lenticularis* males (no. 2 and 3) and one *I. yeyetta* male were re-fixated in 2% osmium tetroxide, CP-dried (BAL-TEC CPD030), sputter-coated (BAL-TEC SCD 005), and observed with Philips SEM 515 (FU Berlin) and JEOL JSM–35 CF (Univ. of Vienna) electron microscopes.

RESULTS AND DISCUSSION

Discovery of *Limnadia lenticularis* Males in Austria

Two *L. lenticularis* males (1, 2) were found in the Morava (March) River flood plain, at the "Lange Lüsse" fields near the village of Schlosshof (48°12′50″N, 16°56′20″E) on 25 June 1995. The third male was collected in a neighboring pool on 20 October 1995. The fourth male was found in the Danube River flood plains, at the "Blumengang" depression near Markthof (48°10′42″N, 16°58′00″E) near the mouth of the Morava River on 15 August 1997 (Fig. 1).

During the years 1994 to 1999, 3,167 specimens of the five Austrian conchostracan species were collected throughout the country. Number of collected specimens and male percentages of each species are given in Table 1.

A more-or-less balanced sex ratio is found in *L. dahalacensis* and *I. yeyetta;* a defini-

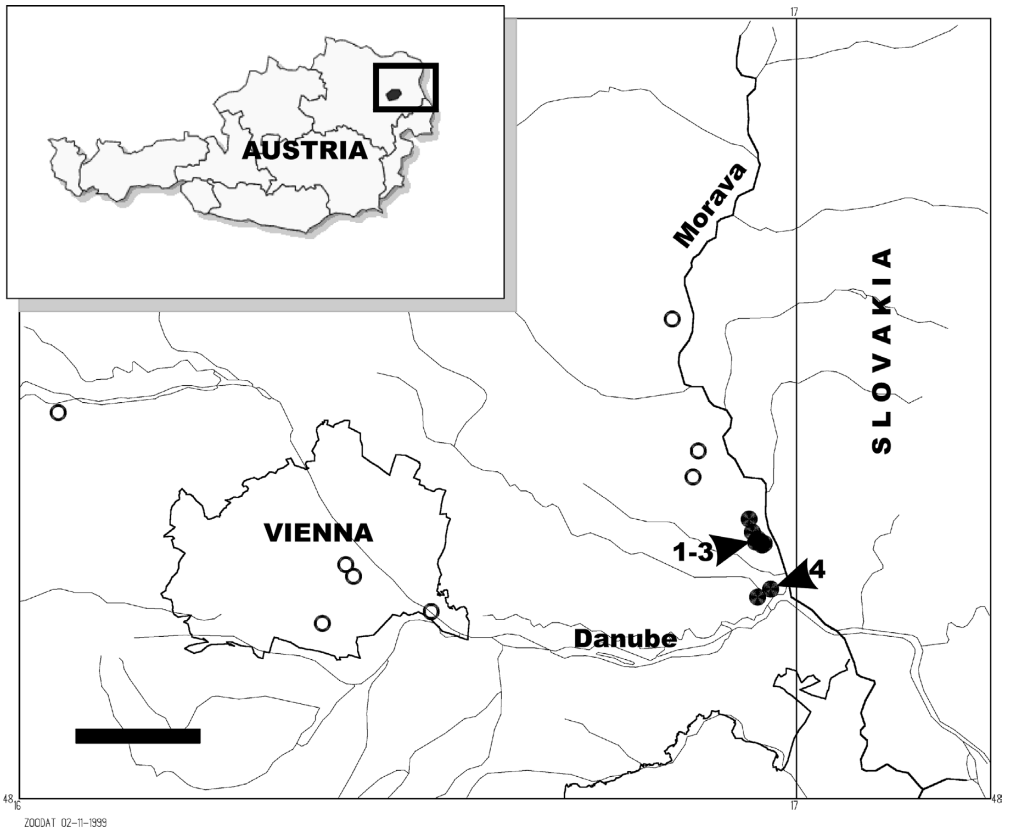

Fig. 1. Records of *Limnadia lenticularis* of the Morava and Danube flood plains in Austria. Records before 1990 (empty circles); records from 1990–1999 (filled circles). Numbers correspond to the males found in 1995 and 1997. Scale bar = 10 km.

tive statement about the sex ratio of *C. tetracerus* and *E. ticinensis* cannot be made due to the low sample numbers. However, in contrast to *L. lenticularis,* males are common in these two species.

Description of *Limnadia lenticularis* Males

Shape and lateral compression of the carapace ("secondary shield" after Walossek, 1993) of *L. lenticularis* males do not differ from those of females (Fig. 2). The carapace valves of the examined *L. lenticularis* males have an average length of 10 mm (n = 3) and a width of 7.3 mm (n = 4; Table 2).

Table 1. Number and sex ratio of conchostracans collected in Austria between 1994 and 1999.

Species	Total number	Male percentage
Cyzicus tetracerus	42	33.3
Imnadia yeyetta	1,452	44.6
Eoleptestheria ticinensis	31	71.0
Leptestheria dahalacensis	1,278	49.8
Limnadia lenticularis	364	1.1

The number of thoracopod-bearing segments in *L. lenticularis* females is reported to be 20–26 (Gruner, 1993). Our *L. lenticularis* males possess 22 thoracopod-bearing segments; the last segment is not clearly separated from the telson. Due to difficulties in segment counting in one male, it cannot be excluded that there is an additional segment in this male. For the description of conchostracan males, the most important structure is the sexual dimorphism of the first two thoracopods, which are modified for clasping during mating (Fig. 2). In the examined *L. lenticularis* specimens, the first two thoracopod pairs were almost identical (Fig. 3A).

These thoracopods consist of a basis with endites, an elongated exopod, and an oval epipod. The proximal endite is oriented at a different angle, its setae are directed toward the

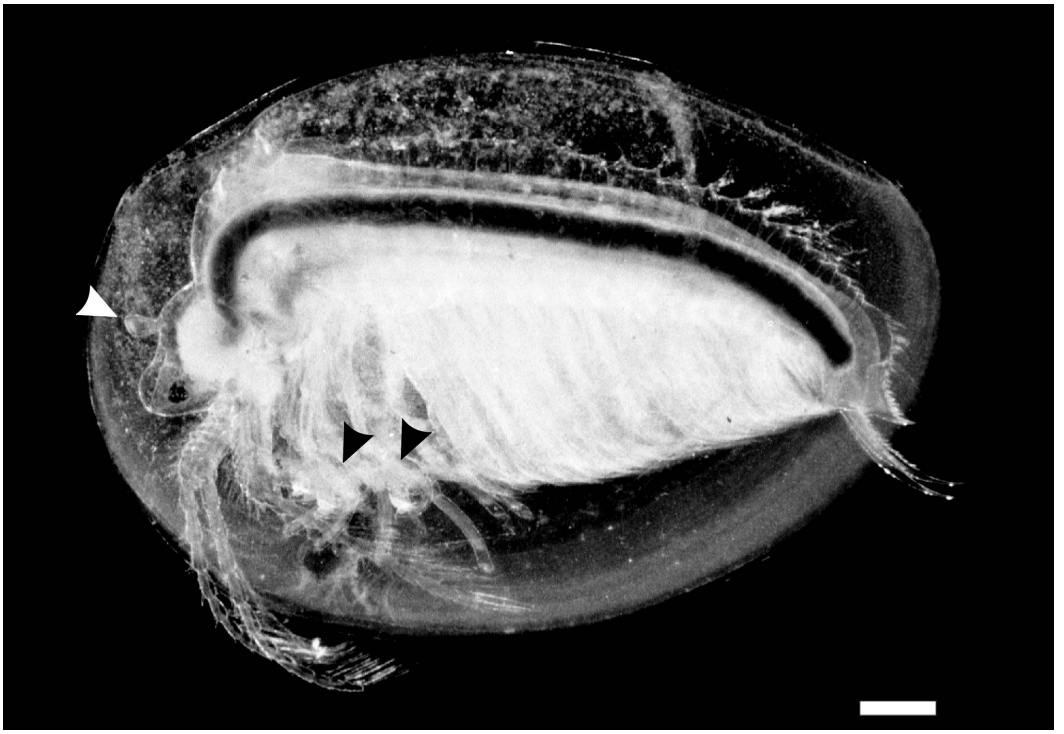

Fig. 2. *Limnadia lenticularis* male 1, alcohol specimen. Arrows indicate the position of male claspers and the pyriform dorsal organ. Scale bar = 1 mm.

midventral food groove. This endite is responsible for the mechanical movement of food particles anteriorly (Martin and Christiansen, 1995). Endites 2 and 3 are almost equal in size and bear rows of plumose setae. Endites 4 and 5 of the protopod, and the endopod, have been modified into the clasping structures (number of endites according to Olesen *et al.*, 1996). The clasper consists of the "palm," the opposing "movable finger," and two palps, a larger and a smaller one. The palm is smooth, without any setae, and of a trapeziform shape that extends toward the distal part (Fig. 3A). A prominent bump-like process can be found on the inner side of the palm. The inner distal face is extended to an "apical club" (Fryer, 1987), distally covered with scale-like short setae (Fig. 3B, C) and long plumose setae (Fig. 3D). The outer distal face is articulated with the opposing movable finger. The movable finger is long and curved with a basal bulge. On its tip it bears a field of small, round teeth (Fig. 3B) opposed to the field of short setae on the apical club, and a sucker-like distal projection (Fig. 3G) reported to be a characteristic of all Limnadiidae (Olesen *et al.*, 1996).

Table 2. Carapace sizes of examined males. The carapace of *L. lenticularis* male 4 was damaged and could not be measured for length.

Specimen	Length (mm)	Width (mm)	Number of growth lines
L. lenticularis male 1	9.5	6.8	3
L. lenticularis male 2	9.2	6.2	3
L. lenticularis male 3	11.4	7.8	4
L. lenticularis male 4	—	8.3	7

Both palps arise from the posterior part of the distal face of the palm. The smaller, one-segmented palp comes off the apical club; the larger, three-segmented palp arises near the articulation of the movable finger. On the tip, both palps possess (probably sensory) setae (Fig. 3E, F); the rest of the palp surface is smooth.

Comparison with *Imnadia yeyetta*

The two central European representatives of Limnadiidae, *L. lenticularis* and *I. yeyetta,* can be distinguished by two main characters, which are valid for both males and females: the dorsal organ is pyriform only in *L. lentic-*

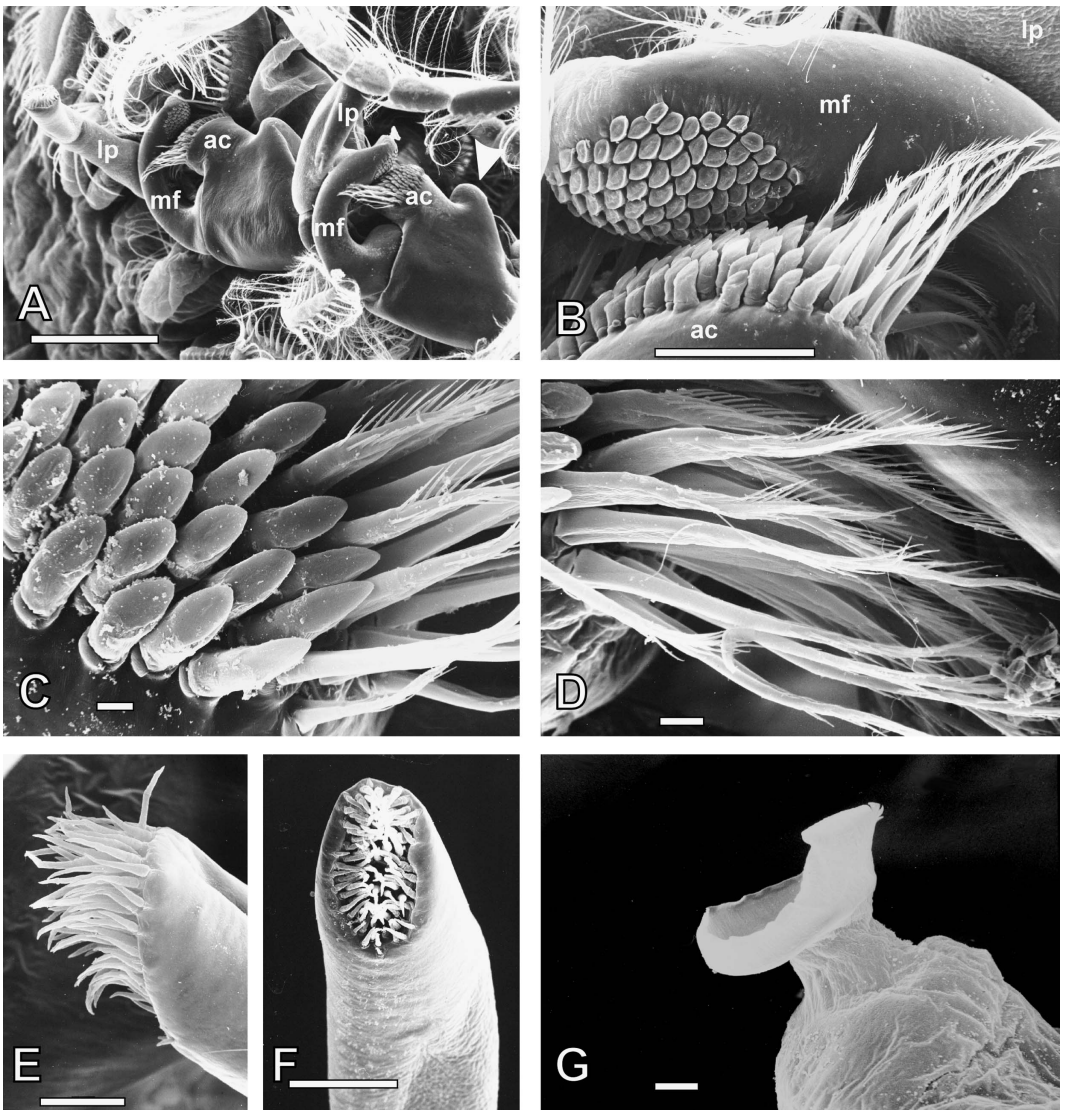

Fig. 3. *Limnadia lenticularis*, male claspers. Abbreviations: ac = apical club, lp = large palp, mf = movable finger. A) Left claspers (male 3), overview. First clasper on the right side, second clasper in the center. Arrow: bump-like process. Scale bar = 500 µm. B) Apical club and opposed movable finger with clasping surface structures (male 3, second clasper). Scale bar = 100 µm. C) Apical club. Short scale-like setae and basis of long plumose setae (male 3, first clasper). Scale bar = 10 µm. D) Apical club. Long plumose setae (male 2, first clasper). Scale bar = 10 µm. E) Large palp, sensory setae, lateral view (male 2, first clasper). Scale bar = 50 µm. F) Large palp, sensory setae, frontal view (male 3, second clasper). Scale bar = 100 µm. G) Distal "sucker" on movable finger (male 2, first clasper). Scale bar = 10 µm.

ularis (Straškraba, 1965: Fig. 2), and the furcae bear minute denticles in *L. lenticularis* vs. long setae in *I. yeyetta*.

The carapace valves of the examined *I. yeyetta* males have an average length of 9.2 mm and an average width of 6.2 mm ($n = 10$).

Imnadia yeyetta males are reported to have 17, females 14, leg-bearing segments (Defaye *et al.*, 1998). However, our investigations of *I. yeyetta* showed 17 thoracopod-bearing segments in both males and females.

Male claspers of *I. yeyetta* (Fig. 4) and *L. lenticularis* can be distinguished by the shorter inner setae of the apical club and the smaller bump-like process of the palm in *I. yeyetta*. A brief comparison of the male

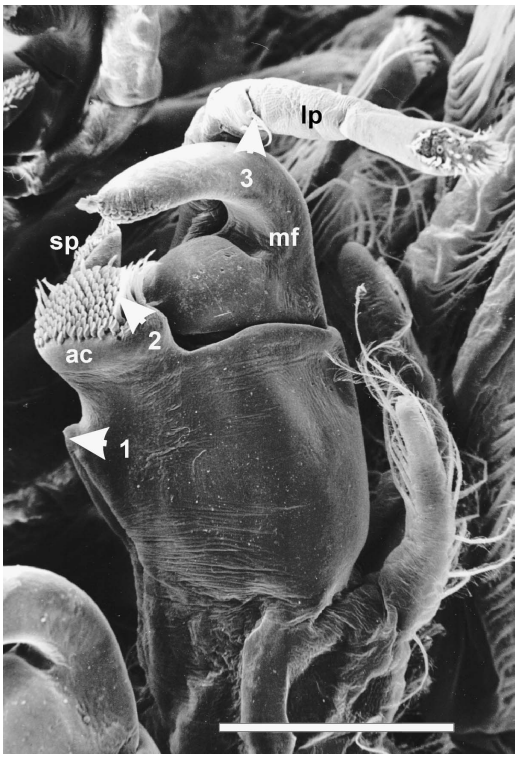

Fig. 4. *Imnadia yeyetta*, second male clasper. Arrow 1: bump-like process; arrow 2: short setae on apical club; arrow 3: projection and setae on large palp articulation. Abbreviations: ac = apical club, lp = large palp, sp = small palp, mf = movable finger. Scale bar = 500 µm.

clasper characteristics between the two species is given in Table 3.

Sex Ratio in Conchostraca

Sassaman (1995: pp. 48–50, table 1) summarized estimated sex ratios of 60 conchostracan species, representing all families and genera. For *E. ticinensis* (n = ?, approx. 50%), *L. dahalacensis* (n = ?, 50–75%), and *I. yeyetta* (n = 32, 56%), he gives a male percentage which exceeds 50%. For *C. tetracerus,* Sassaman (1995), referring to Mathias (1937), gives a male/female ratio of 0.28 (n = 1,364). This corresponds to our data (Table 1) and is definitively lower than in the aforementioned three species. However, for these four species, obligatory sexual reproduction can be assumed.

In contrast to the conchostracan species mentioned above, males are extremely rare in *L. lenticularis,* which is, according to Sassaman (1995: p. 50), "a species in which previous collections over the last century and a half (and totaling thousands to tens of thousands of individuals) have not contained any males." In light of this very extensive earlier sampling, we suggest that the four newly recorded males are rare exceptions and do not represent a constant small number of males in the European populations. This is also supposed for the two *L. lenticularis* males found by J. W. Martin in a population in Florida, U.S.A., in 1991 (Sassaman, 1995).

The new records of males disprove the exclusive parthenogenesis in *L. lenticularis* as it was reported by several authors (Sars, 1896; Gislén, 1937; Mathias, 1937; Defaye *et al.*, 1998). Accepting the genotype proposal by Sassaman (1995) and considering the spontaneous occurrence of males, then scarce selfing in the sexually heterozygous (S/s genotype) "females" has to be supposed. Male gonads found in phenotypic females by Zaffagnini (1969), albeit called "rudimentary," show that selfing is a possible mode of reproduction in *L. lenticularis*. Therefore, *L. lenticularis* may be facultatively capable of parthenogenesis or selfing in the absence of males (Zaffagnini, 1969), and of outcrossing (cross-fertilization?) in the presence of males, although the latter can only be supposed without further evidence.

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Table 3. Morphological comparison of male claspers in the European Limnadiidae.

Character	*L. lenticularis* (Fig. 3A–G)	*I. yeyetta* (Fig. 4)
Large palp	sensory setae on the top, articulation smooth	sensory setae on the top, articulation bearing setae and projection
Bump-like process	large, prominent	small
Apical club	long plumose inner setae	short inner setae

us the opportunity to work on the SEM. We thank M. Malicky (ZOODAT, Linz) for friendly cooperation. Last, but not least, we acknowledge the supportive comments on this work by K. Haas, W. Klepal, J. W. Martin, J. Olesen, G. Scholtz, and an anonymous referee.

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