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Molecular phylogeny of the genus *Lythria* and description of the male genitalia of *L. venustata* (Lepidoptera: Geometridae: Sterrhinae)

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Abstract. We present a molecular phylogeny incorporating all five species of the Palaearctic geometrid genus *Lythria*, based on a 2810-bp combined data matrix comprising the full sequence of the mitochondrial gene cytochrome oxidase subunit one (COI) and fragments of the nuclear genes elongation factor 1 alpha (EF-1α) and wingless (wgl). *L. venustata*, which was recently rediscovered from Kazakhstan, is shown to be sister taxon to all other members of the genus. The remaining species within the genus form two pairs of sister species: *L. purpuraria* groups together with *L. plumularia*, and *L. cruentaria* with *L. sanguinaria*. The phylogeny is well supported by characters of the male genitalia of all *Lythria* species. In addition to the molecular phylogeny of the genus *Lythria*, we illustrate the external appearance of *L. venustata* for the first time and describe the anatomy of its male genitalia.

INTRODUCTION

The systematic placement of the Palaearctic geometrid genus *Lythria* Hübner, 1823 has long been problematic (Hausmann, 2004; Sihvonen & Kaila, 2004). Though usually regarded as a larentiine taxon since Meyrick (1892), a position within Sterrhinae has also been proposed (Pierce, 1914). Recently, a molecular phylogenetic study by Õunap et al. (2008) demonstrated that *Lythria* indeed belongs within Sterrhinae, and that several morphological characters linking *Lythria* with Larentiinae are possibly plesiomorphic in the Sterrhinae.

Lythria comprises five species, all of which occur in the Palaearctic (Scoble, 1999; Viidalepp, in press). Two of them, *L. purpuraria* (Linnaeus, 1758) and *L. cruentaria* (Hufnagel, 1767), are widely distributed, from Western Europe to Central Asia (Häuser, 2001; Viidalepp, in press). Historically, there has been a lot of confusion between *L. purpuraria* and *L. cruentaria,* since both species exhibit considerable variation in size and wing pattern. Separation of these species on the basis of external morphology is therefore often difficult. Consequently, these taxa were usually treated as a single species, *L. purpuraria* (but see, e.g., Borkhausen, 1794; Laspeyres, 1803; Duponchel, 1830), until Prout (1914) noted that the taxon in fact comprises two species that are easily distinguishable on the basis of genital characteristics. Subsequently, the genitalia of *L. purpuraria* and *L. cruentaria* [the synonym *L. purpurata* (Linnaeus, 1761) being widely used in the older literature] have been illustrated repeatedly (e.g., Zerny, 1916; Błeszyński, 1965; Häuser, 2001), and differences in the distribution and life-history traits of these species have been well documented (e.g., Koch, 1984; Müller, 1996; Häuser, 2001; Viidalepp, 1996).

The third species, *L. sanguinaria* (Duponchel, 1842), occurring only in the Iberian Peninsula and in southern France (Prout, 1914; Viidalepp, in press) was treated as a separate species for a long time (see, e.g., Staudinger & Rebel, 1901; Spuler, 1903–10). However, both Prout (1914) and Zerny (1916) found that the differences between the genitalia of *L. cruentaria* and *L. sanguinaria* were insufficient to regard them as distinct species. Since their studies, the latter has been treated as a subspecies of *L. cruentaria* (Prout, 1937; Herbulot, 1962; Leraut, 1997; Scoble, 1999). Recently, Viidalepp (in press) showed that there are limited but consistent differences between the taxa in the wing pattern and both in male and female genitalia. He therefore proposed reinstating species status to *L. sanguinaria.* A subsequent study by Õunap et al. (2008) revealed that the level of genetic divergence between *L. sanguinaria* and *L. cruentaria* is almost as large as that between *L. cruentaria* and *L. purpuraria*, thus implicitly supporting the view of Viidalepp (in press) that *L. sanguinaria* is a distinct species.

The fourth species, *L. plumularia* (Freyer, 1831), is an endemic of the European High Alps (Prout, 1914; Viidalepp, in press). Though *L. plumularia* is also extremely variable in appearance (Gradl, 1938), it nevertheless is morphologically clearly distinct from the taxa discussed above. Confusion between *L. plumularia* and other European taxa is therefore highly unlikely. The genitalia of this species have recently been illustrated for the first time (Vasilenko, 2009).

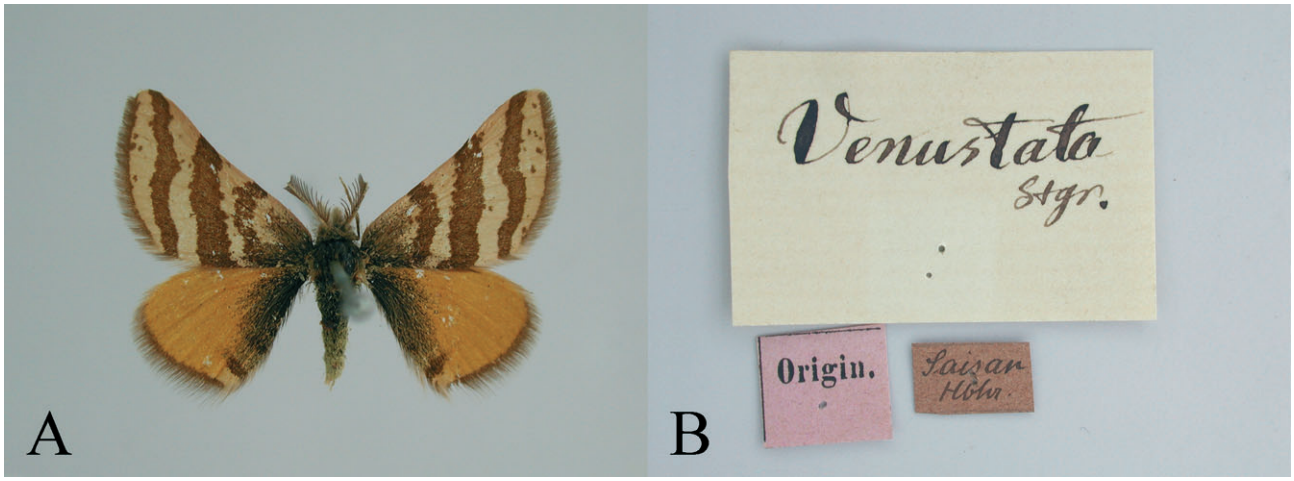

Fig. 1. A – holotype of *Lythria venustata*, coll. MNHU. Wingspan 16 mm; B – original labels.

The fifth species, *L. venustata* Staudinger, 1882, is the least well known. For more than a century, only the holotype (male) was known. The specimen was collected from Zaisan in eastern Kazakhstan and is currently housed at the Museum für Naturkunde, Humboldt-Universität, Berlin, Germany (Fig. 1). Although *L. venustata* was included in the major monographs of Palaearctic Geometridae by Staudinger & Rebel (1901) and Prout (1914), these did not include illustrations of the species; neither have illustrations appeared elsewhere. Recently, two additional *L. venustata* males were collected, one from a lowland semidesert in western Kazakhstan in 2006 (Fig. 2), another from Tarbagatai mountains in northeastern Kazakhstan in 2007 (Vasilenko, 2009). On the basis of the latter, Vasilenko (2009) illustrated the male genitalia of *L. venustata* for the first time. Unfortunately, he did not spread the heavily sclerotized valvae. This resulted in misinterpretations regarding the development and position of some genitalic structures.

The complete phylogeny of *Lythria* has hitherto not been studied. However, on the basis of external morphological characters, the moths fall into two groups. First, *L. plumularia* and *L. venustata*, which have ochreous-yellow forewings with three heavily contrasting dark transverse fasciae, are assumed to be closely related (Staudinger, 1882; Prout, 1914; Vasilenko, 2009). Second, *L. purpuraria*, *L. cruentaria* and *L. sanguinaria*, which typically have greenish-yellow forewings with two (*L. purpuraria*) or three (*L. cruentaria*, *L. sanguinaria*) purple red transverse lines, are believed to form another group. Within the latter group, both external characters and the male and female genitalia suggest that *L. cruentaria* and *L. sanguinaria* represent sister taxa (Viidalepp, in press). Surprisingly, Õunap et al. (2008) found *L. sanguinaria* and *L. purpuraria* to be very closely related, with *L. cruentaria* appearing as sister taxon to them. However, the authors of this study noted that their intrageneric phylogeny may have been influenced by homoplasy in their data matrix (Õunap et al., 2008).

Thus, the phylogeny of *Lythria* including all known species (neither *L. plumularia* nor *L. venustata* have been incorporated into previous molecular phylogenetic analyses) still remains to be determined. For this purpose, the full sequence of mitochondrial gene cytochrome oxidase subunit 1 (*COI*) and partial sequences of two nuclear genes [elongation factor 1 alpha (*EF-1α*) and wingless (*wgl*)] were sequenced for all five *Lythria* species and used to construct the phylogeny of the genus. The male

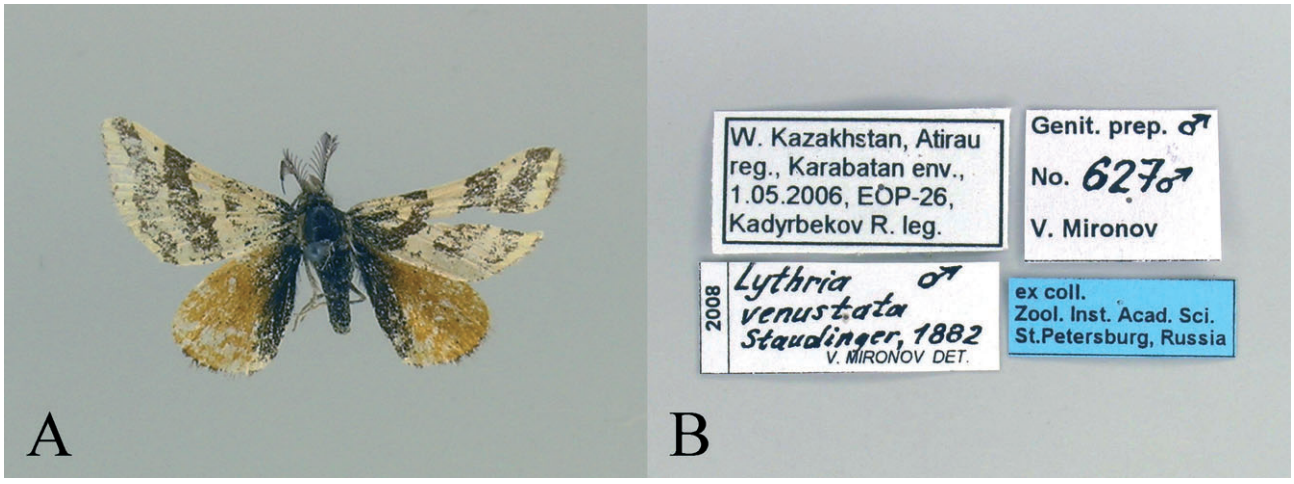

Fig. 2. A – second known *Lythria venustata* specimen, coll. ZISP. Wingspan 16 mm; B – original labels.

TABLE 1. Information on the specimens used in molecular analyses. Collecting site (ESP – Spain, EST – Estonia, KAZ – Kazakhstan, SUI – Switzerland) and date, collector's name, GenBank accession numbers for *COI*, *EF-1α* and *wingless* sequences and depositories of the studied specimens are indicated. Authorships of the sequences downloaded from GenBank are indicated as follows: ¤ – Knölke et al., 2005; # – Õunap et al., 2008.

Species	Collecting site	Date	Collector	*COI*	*EF-1α*	*wingless*	Depository
Timandra griseata Petersen, 1902	EST, Tartumaa, Tatra valley near Kambja	02.07.2003	E. Õunap	EU443362#	EU443299#	EU443319#	Coll. IZBE
Timandra comae Schmidt, 1931	EST, Tartumaa, Tatra valley near Kambja	11.09.2003	E. Õunap	EU443363#	EU443300#	EU443320#	Coll. IZBE
Rhodometra sacraria (Linnaeus, 1767)	EST, Pärnumaa, centre of Nigula Nature Reserve	23.08.2000	M. Kruus	AJ870398¤	EU443305#	EU443325#	Coll. IZBE
Lythria purpuraria (Linnaeus, 1758)	ESP, Barcelona 50 km N, Sant Pere de Vilamajor	21.08.1999	T. Tammaru	EU443367#	EU443304#	EU443324#	Coll. T. Tammaru
Lythria cruentaria (Hufnagel, 1767)	EST, Harjumaa, Põhja-Kõrvemaa Landscape Reserve, Jussi heath	29.06.2004	E. Õunap	EU443365#	EU443302#	EU443322#	Coll. IZBE
Lythria sanguinaria (Duponchel, 1842)	ESP, (MA) Tres Cantos 740 m	15.05.2006	G. King	EU443366#	EU443303#	EU443323#	Lost in mail
Lythria plumularia (Freyer, 1831)	SUI, Graubünden Albula-Pass 1,800 m. TF	20.06.2005	R. Baumberger	GQ857123	GQ857125	GQ857127	Coll. N. Pöll
Lythria venustata Staudinger, 1882	KAZ, W Kazakhstan, Atirau reg., Karabatan env.	01.05.2006	R. Kadyrbekov	GQ857124	GQ857126	GQ857128	Coll. ZISP

genitalia of *L. venustata* were illustrated for the second time and their anatomy discussed and compared to the male genitalia of the other *Lythria* species.

MATERIAL AND METHODS

Moths

In total, eight species were analyzed (Table 1). In addition to the five *Lythria* species, *Rhodometra sacraria* (Linnaeus, 1767) from the tribe Rhodometrini, which is currently believed to be the closest relative to the Lythriini (Õunap et al., 2008), was also included in the analysis, while *Timandra griseata* Petersen, 1902 and *T. comae* Schmidt, 1931 were used as outgroups. Details of collecting data and the depositories of voucher specimens are presented in Table 1.

DNA extraction, amplification and sequencing of gene fragments

Total genomic DNA was extracted from the anterior abdominal segments (for details, see Õunap et al., 2005) or from two or three legs of individual specimens. Purification of total genomic DNA was performed using a High Pure PCR Template Preparation Kit (Roche Diagnostics GmbH, Mannheim, Germany) following the manufacturer's instructions for isolating nucleic acids from mammalian tissue, except that the first incubation step was 55°C for 2–3 h rather than for 1 h.

Two partially overlapping fragments were amplified using two main primer pairs (cov1f+nan and v1+4r2, Table 2) to obtain the full sequence of the *COI* gene. However, PCR amplification using these primers failed in some taxa. Therefore, additional primer pairs, cov1f+cov1r, ron+nan, v1+v2 and 4f2+4r2 (Table 2), were used to amplify *COI* in four shorter partially overlapping fragments. A partial sequence of the *wgl* gene was amplified using primers LepWG1+LepWG3, while a fragment of *EF-1α* was amplified using primers ef44 and efrcM4 (Table 2). As was the case for *COI*, the latter primers did not perform well in some taxa, so additional primer combinations, LepEF-1f+LepEF-1r, EF51.9+Niina2 and LepEF-2f+Niina2 (Table 2), were used to amplify *EF-1α* in two shorter partially overlapping fragments. PCR was performed in a total volume of 20 µl, with the reaction mixture containing 1X BD Advantage 2 PCR buffer, 1U BD Advantage 2 Polymerase mix (BD Biosciences, San Jose, USA), 0.2 mM dNTP (Fermentas, Vilnius, Lithuania), 4 pmol of primers and 20–80 ng of purified genomic DNA.

PCR was performed using a T1 thermocycler (Biometra, Göttingen, Germany) with the following cycling parameters: A 2 min denaturing step at 94°C followed by 35–40 cycles of 30 s at 94°C, 30 s at various temperatures, depending on primers (Table 2) and 60–75 s at 68°C with a subsequent 7-min final extension at 68°C PCR products were visualised on a 1.6% agarose gel, and 10 µl of the PCR solution was treated with shrimp alkaline phosphatase and exonuclease I (Fermentas, Vilnius, Lithuania). One unit of each enzyme was added to the PCR solution, which was incubated for 27 min at 37°C followed by 15 min inactivation at 80°C.

DNA cycle sequencing was performed in a total volume of 10 µl using the DYEnamic ET Terminator Cycle Sequencing Kit (GE Healthcare, Chalfont St Giles, United Kingdom). Cycling conditions were: 33 cycles of 20 s at 95°C, 20 s at various temperatures, depending on the primer (Table 2), and 60 s at 60°C. Both DNA strands were sequenced using 5 pmol of primers, and sequences were resolved using an ABI PRISM 377 automated sequencer (Applied Biosystems, Foster City, USA).

Phylogenetic analysis

Consensus sequences were created in Consed (Gordon et al., 1998), using sequence data from both DNA strands. Sequences were double-checked by eye, edited in BioEdit (Hall, 1999) and aligned in ClustalW using the default settings (Thompson et al., 1994). Homogeneities between all gene sequences were calculated using the partition homogeneity test in PAUP*4.0b10 (Swofford, 1998). This test was performed with the random addition heuristic search option, using 1,000 replicates. As no incongruence between different genes was found, the whole data matrix was subsequently analysed as a single entity. Modeltest 3.06 (Posada & Crandall, 1998) was used to search for the optimal model of DNA substitution. Bayesian phylogenetic inference, maximum likelihood (ML), maximum parsimony (MP) and neighbour-joining (NJ) approaches were all used to evaluate the robustness of the phylogenetic analysis.

The GTR+I+Γ model, selected by Modeltest, was used for NJ and ML analysis in PAUP. Branch supports were assessed using 1,000 bootstrap replicates. MP analysis with simple addition of

TABLE 2. Details of primers and PCR and cycle sequencing reactions. PCR and CS indicate the temperature during the annealing step of PCR amplification and cycle sequencing, respectively. Primer Verdi4 was used only as an internal primer for sequencing the 5' half of EF-1α.

Primer	Primer sequence	Gene region	Direction	PCR	CS	Source
Cov-1f	5'-TCG CTT ATT ATT CAG CCA TTT TAT T-3'	COI, 5' half	Forward	50°C	47°C	Õunap et al., 2008
Cov-1r	5'-CTG CAC CAT TTT CTA CAA TTC TTC T-3'	COI	Reverse	50°C	50°C	Õunap et al., 2008
Ron	5'-GGA TCA CCT GAT ATA GCA TTC CC-3'	COI	Forward	49°C	53°C	Caterino & Sperling, 1999
Nan	5'-CCC GGT AAA ATT AAA ATA TAA ACT TC-3'	COI, 5' half	Reverse	49–50°C	47°C	Õunap et al., 2005
V1	5'-ATA TTA TTA ACW GAT CGA AAY TTA AAT AC-3'	COI, 3' half	Forward	45–50°C	47°C	Õunap et al., 2008
V2	5'-TGA AAA TGA GCT ACW ACA TAA TAA GTA TCA-3'	COI	Reverse	50°C	45°C	Õunap et al., 2008
4f2	5'-ATT AAA ATT TTT AGT TGA TTA GC-3'	COI	Forward	50°C	45°C	Õunap et al., 2008
4r2	5'-CTT TAT AAA TGG GGT TTA AAT C-3'	COI, 3' half	Reverse	45–50°C	47°C	Õunap et al., 2008
LepWG1	5'-GAR TGY AAR TGY CAY GGY ATG TCT GG-3'	Wingless	Forward	58°C	55°C	Brower & DeSalle, 1998
LepWG3	5'-ACT YCG CAR CAC CAR TGG AAT GTR CA-3'	Wingless	Reverse	58°C	55°C	Brower & DeSalle, 1998
ef44	5'-GCY GAR CGY CAR CGT GGT ATY AC-3'	EF-1α	Forward	58°C	58°C	Monteiro & Pierce, 2001
efrcM4	5'-ACA GCV ACK GTY TGY CTC ATR TC-3'	EF-1α	Reverse	58°C	58°C	Monteiro & Pierce, 2001
LepEF-1f	5'-AAR TAC TAT GTC ACN ATC ATY GA-3'	EF-1α, 5' half	Forward	55°C	55°C	Õunap et al., 2008
Verdi4	5'-CAC CAG TCT CCA CAC GGC C-3'	EF-1α, 5' half	Reverse		57°C	Viidalepp et al., 2007
LepEF-1r	5'-ACA CCA GTT TCN ACW CKG CC-3'	EF-1α, 5' half	Reverse	55°C	55°C	Õunap et al., 2008
EF51.9	5'-CAR GAC GTA TAC AAA ATC GG-3'	EF-1α, 3' half	Forward	58°C	57°C	Monteiro & Pierce, 2001
LepEF-2f	5'-CCC ACA GAC AAG SCY CTV CGT-3'	EF-1α, 3' half	Forward	61°C	55°C	Õunap et al., 2008
Niina2	5'-CCT GGA AGG ACT CCA CRC ACA G-3'	EF-1α, 3' half	Reverse	58–61°C	57°C	Viidalepp et al., 2007

taxa was also performed in PAUP and resulted in a single most parsimonious tree. Branch supports for this tree were assessed using 1,000 bootstrap replicates, with 10 heuristic searches and simple addition of taxa used for each replicate. ML, NJ and MP trees were visualised in TreeView 1.6.6 (Page, 1996).

Bayesian phylogenetic analysis implementing the GTR+I+Γ model was performed using MrBayes 3.1 (Ronquist & Huelsenbeck, 2003). Four simultaneous Markov chains (one cold and three heated) were run for 2,000,000 generations, with trees sampled every 1,000 generations. Likelihood values were inspected, and the first 500 sampled trees were discarded as "burn-in". To estimate posterior probabilities of recovered branches, a 50% majority rule was applied. Phylograms were created as average-branch-length consensus trees and visualised in TreeView 1.6.6.

RESULTS AND DISCUSSION

All three gene fragments were successfully sequenced for all analysed specimens. The total length of the *COI* gene was 1536 bp for all *Lythria* species and *R. sacraria*, while both *Timandra* species had an 8-bp insertion (AAAAATAT) between the COI positions 1531 and 1532. The total length of the *COI* gene was therefore 1533 bp for the *Timandra* species, as the 8-bp insertion resulted in the formation of a TAA stop codon in *COI* positions 1531–1533. The length of the successfully sequenced fragment of *EF-1α* gene was 883 bp, while the length of the successfully sequenced *wgl* fragment was 383 bp. No insertions or deletions were identified in either of these nuclear genes. The total length of the combined molecular data matrix was 2810 bp.

All phylogenetic analyses resulted in well-resolved trees that exhibited identical topology and maximal or near-maximal indices of support for all nodes (Fig. 3). *R. sacraria* was placed as the closest relative to the genus *Lythria*, while *L. venustata* appeared as sister taxon to all other *Lythria* species. Four remaining *Lythria* species formed two clades of sister taxa: *L. purpuraria* was placed together with *L. plumularia* and *L. cruentaria* with *L. sanguinaria* (Fig. 3).

Lythriini were postulated as the sister group to the Rhodometrini by Õunap et al. (2008) on the basis of molecular data. In addition, they listed a few morphological and ecological characters that link Lythriini with Sterrhinae in general, and support the tribe's position as sister to the Rhodometrini. Similarly, Sihvonen & Kaila (2004) found a close association between *Lythria* and *Rhodometra* Meyrick, 1892. This confirms the finding of Õunap et al. (2008) that the genus belongs to the Sterrhinae. A synapomorphy for the Timandrini lineage sensu Sihvonen & Kaila (2004), the absence of sensilla on the ventral surface of the male flagellomere, is also characteristic of *Lythria*.

Additionally the following four synapomorphies, from a total of seven found to be characteristic of the Rhodometrini by Sihvonen & Kaila (2004), are also found in

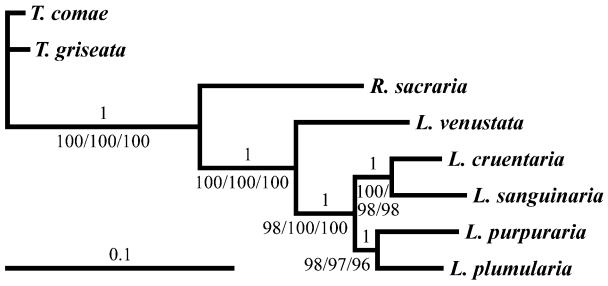

Fig. 3. Bayesian phylogenetic tree (GTR+I+Γ model) of the genus *Lythria*, based on a 2810 bp combined sequence of *COI*, *EF-1α* and *wgl* sequences. Bayesian posterior probabilities are given above the branches, bootstrap support for the ML/NJ/MP trees, which exhibited identical topology, are presented below the branches.

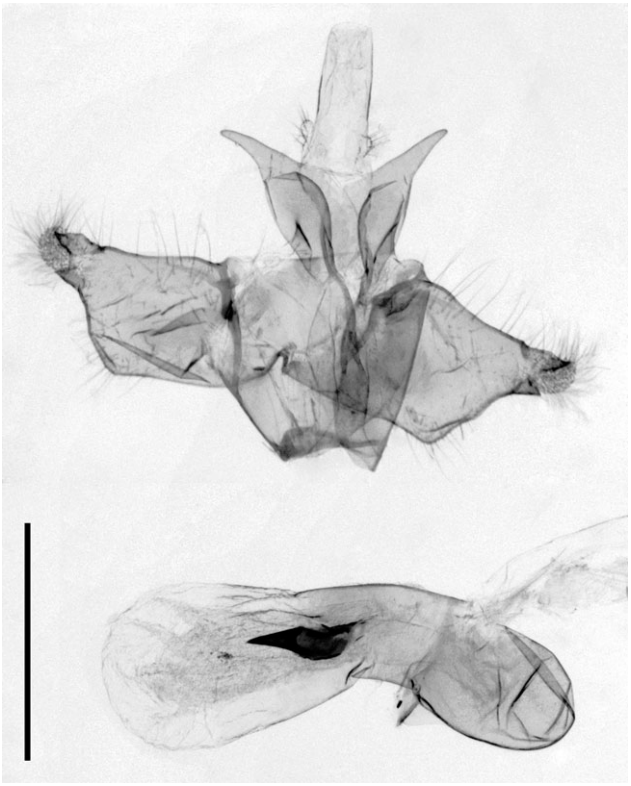

Fig. 4. Male genitalia of *Lythria purpuraria*: Estonia, gen. prep. 8130 J. Viidalepp, coll. Petersen/IZBE. Scale bar: 1 mm. MorphBank accession code 478410.

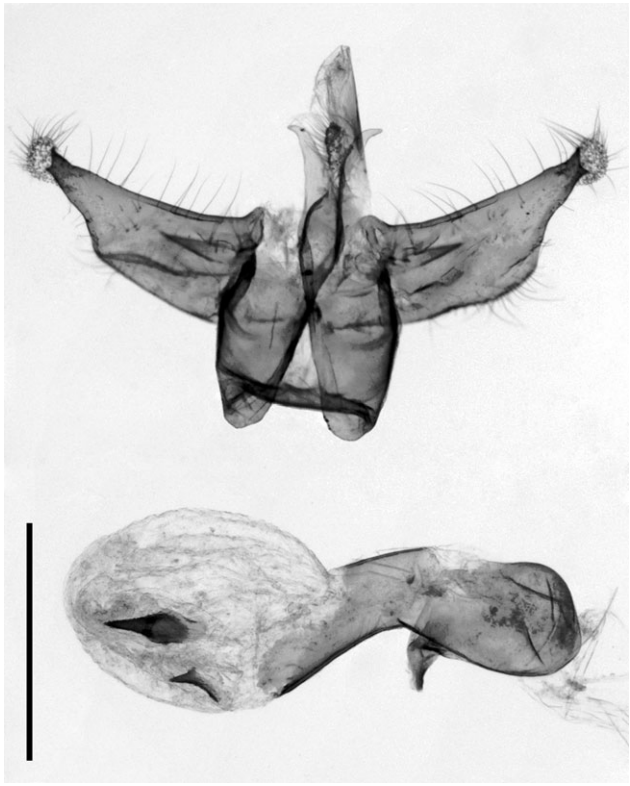

Fig. 5. Male genitalia of *Lythria plumularia*: Switzerland, Davos, coll. NHRS. Scale bar: 1 mm. MorphBank accession code 478411.

the Lythriini: terminal line of the forewings absent; hindwing veins Sc and R_1 fused for a relatively long distance (also noted by Õunap et al., 2008); uncus naked; and arms of transtilla do not meet dorsally. The remaining three characters (Sihvonen & Kaila, 2004), however, are absent in Lythriini: reddish straight line from the forewing apex; forewing ground colour bright yellow; and spinose apex of sacculus. The distinctive shape of ansa, which was listed as an additional character of Rhodometrini by Sihvonen & Kaila (2004), is not found in Lythriini. The central flap of the ansa is strongly dilated unilaterally in Rhodometrini but only slightly broadened in Lythriini.

Additional common characteristics that support the proposed sister relationship between the Rhodometrini and Lythriini are the following: Large vinculum (resembling that of Scopulini; see Sihvonen & Kaila, 2004), weak tegumen, weak juxta, absence of saccus and the presence of a pair of pad-like socii on the base of the uncus in *Casilda* Agenjo, 1952 and *Lythria*. Socii in *Rhodometra* are, however, as strongly sclerotized as tegumen is.

Lythria has been separated into a tribe of its own, Lythriini, by Herbulot (1962). Its tribal status is justified due to its distinctive external appearance and unique genital morphology (Błeszyński, 1965; Viidalepp, in press). Important male genitalia synapomorphies in *Lythria* species are as follows: (1) postero-lateral extensions of the tegumen; (2) a basal approximation of valvae (i.e., the bases of the sacculi are fused medially); (3) a membranous fultura inferior (i.e., juxta not developed, or weak); and (4) the transition between the heavily sclerotized valva and papillose valvula is well-defined (Figs 4–7).

On the basis of male genital morphology it is possible to distinguish three groups within *Lythria*. First, *L. purpuraria* and *L. plumularia*, though superficially very different, share the presence of two cornuti on the vesica, the presence of a pair of long postero-lateral extensions on the tegumen and a short sack-like, globular valvula attached to the roughly triangular valva (Figs 4–5). In both species, the valvula is approximately as long as it is broad. Second, *L. cruentaria* and *L. sanguinaria* have short, roughly rectangular valvae with long membranous valvulae, short postero-lateral extensions on the tegumen and the presence of one cornutus on the vesica. In these species, the valvula is approximately four times longer than it is broad, though it is clearly more slender in *L. sanguinaria* than in *L. cruentaria* (Figs 6–7). Third, although *L. venustata* is externally quite similar to *L. plumularia* [which obviously led Staudinger (1882), Prout (1914) and Vasilenko (2009) to the conclusion that these species are closely related], its male genitalia are strikingly different from those of the other *Lythria* species. The valvae of *L. venustata* are distally bipartite, the valvulae are absent and the remnants of the socii are missing. The postero-lateral extensions of the tegumen are visible but are much shorter than those in other *Lythria* species. The rounded shape and massive sclerotization of the vinculum gives the genital armature a distinctive appearance (Fig. 8). Vasilenko (2009) described the male genitalia of

Fig. 6. Male genitalia of *Lythria cruentaria*: Estonia, gen. prep. 8128 J. Viidalepp, coll. IZBE. Scale bar: 1 mm. MorphBank accession code 478412.

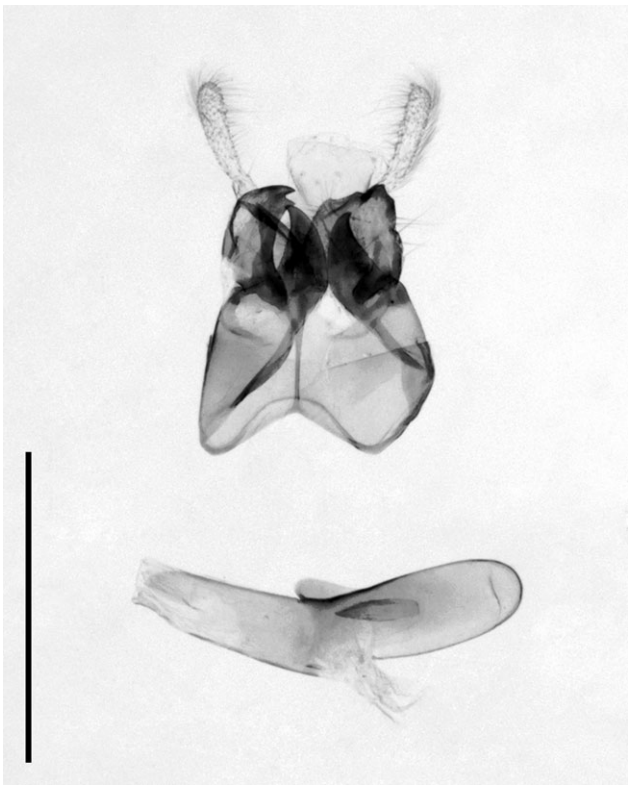

Fig. 7. Male genitalia of *Lythria sanguinaria*: Spain, vic. Madrid, gen. prep. 8127 J. Viidalepp, coll. IZBE. Scale bar: 1 mm. MorphBank accession code 478413.

L. venustata as having long finger-like socii on the posterior edge of the tegumen. This misinterpretation apparently has happened because he did not spread the tough and strongly sclerotized valvae. These "socii" are actually the projections of the sacculi. Furthermore, in Vasilenko's (2009) interpretation *L. venustata* also lacks an uncus and the most distal part of the genitalic capsule is instead the anellus. In contrast, we regard the most distal part of the genitalic capsule as a weakly sclerotized uncus, as seen in the lateral view of the male genitalia (Fig. 8). The aedeagus of *L. venustata* is more slender than that of *L. purpuraria* and *L. plumularia*, but resembles the slim aedeagus of *L. cruentaria* and *L. sanguinaria*. In common with *L. cruentaria* and *L. sanguinaria*, *L. venustata* has one cornutus on the vesica; however, in contrast to all the other *Lythria* species, *L. venustata* has a well developed juxta.

Thus, the results of the molecular phylogenetic analysis are in concordance with those derived from examination of the genital morphology of *Lythria* males. *L. venustata*, whose genital morphology is dissimilar to all other *Lythria* species, was placed phylogenetically as sister taxon to all other species in the genus (Fig. 3). Interestingly, such a position receives additional support from morphological similarities shared by *L. venustata* and *R. sacraria*. Specifically, these two taxa both exhibit a rounded vinculum, which contrasts with the strongly emarginated vinculum of the other *Lythria* species. Moreover, the valva of *R. sacraria*, which is slightly bilobed in shape, also somewhat resembles that of *L. venustata* (see Hausmann, 2004), while the very short postero-lateral extensions on the tegumen of *L. venustata* appear intermediate between Rhodometrini (no such extensions) and other *Lythria* species (well-developed extensions). Sister-taxa relationships between *L. purpuraria* and *L. plumularia*, and *L. cruentaria* and *L. sanguinaria*, respectively (Fig. 3), revealed by the phylogenetic analysis, are also supported by the examination of genital morphology (Figs 4–7). On the other hand, the preliminary subdivision of *Lythria* into two groups on the basis of wing colour (see above) is not supported by the results of the phylogenetic analysis. Though both *L. venustata* and *L. plumularia* have ochreous-yellow forewings in contrast to the other three species, they do not pair as sister taxa (Fig. 3). Earlier hypotheses by Staudinger (1882), Prout (1914) and Vasilenko (2009), which assumed close affinities between *L. venustata* and *L. plumularia*, are therefore rejected, and their superficial similarity is to be regarded as a plesiomorphic or homoplasic condition. Similarly, the sister-taxa relationship between *L. sanguinaria* and *L. purpuraria* proposed by Õunap et al. (2008) must be rejected.

In summary, *L. venustata* appears to be clearly distinct from other *Lythria* species on the basis of both molecular phylogenetic and morphological evidence. Considering the extent of the differences between the male genitalia of *L. venustata* and other *Lythria* species, it may be appropriate to move the former into a genus of its own, as already pointed out by Vasilenko (2009). However, as a female *L. venustata* has yet to be described, we prefer not

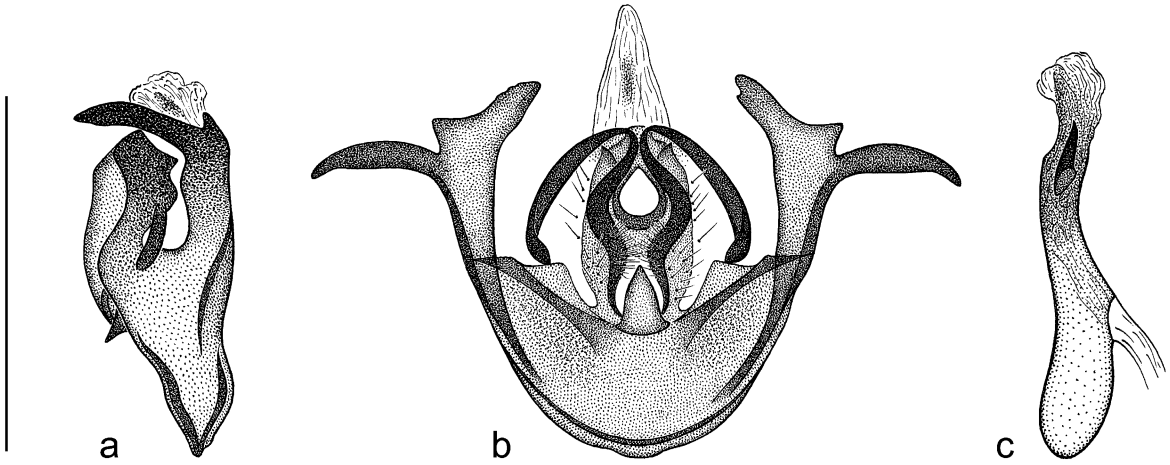

Fig. 8. Male genitalia of *Lythria venustata*. A – lateral view; B – ventral view; C – aedeagus. Scale bar: 1 mm. MorphBank accession code 478414.

to take this step. Nonetheless, we highlight this as a point for consideration in future studies.

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