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Species delimitation, classical taxonomy and genome skimming: a review of the ground beetle genus *Lionepha* (Coleoptera: Carabidae)

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The western North American genus *Lionepha* is shown to contain at least 11 species through a combination of eight-gene species delimitation analyses and morphological study. In order to confirm the names of several species, we sequence DNA of primary types of several names, including a LeConte lectotype collected in the 1850s, using next-generation sequencing. We examine chromosomes of eight species, and show that all have 12 pairs of autosomes and an X0/XX sex-chromosome system. The following species are described as new: ***Lionepha australerasa***, ***L. kavanaughi***, ***L. lindrothi*** and ***L. tuulukwa***. The name *Lionepha erasa* is shown to belong to a relatively rare, western species ranging from Oregon through Alaska; the common, widespread species previously known as *Lionepha erasa* now takes the name *L. probata*. *Bembidion chintimini*, *B. lindrothellus* and *B. lummi* are synonymized with *L. erasa*. We provide tools to identify specimens to species, including illustrations, diagnoses and distribution maps.

ADDITIONAL KEYWORDS: Trechinae, Bembidiini, species delimitation, chromosomes, ancient DNA, ground beetle

INTRODUCTION

Projects in systematic entomology often begin through serendipitous discoveries made in the course of other pursuits. On 12 September 2009, a few days after having moved to Corvallis, Oregon, the first author drove up nearby Marys Peak in search of *Bembidion chintimini* Erwin & Kavanaugh, 1981 (now in the genus *Lionepha*), a species at the time known only from a single female from the summit of the mountain. There was no expectation that any taxonomic research would be done on *Lionepha* as a result, as the group had been revised by Erwin & Kavanaugh (1981); the search was conducted only with the hope of rediscovering a rare species. However, determining if the specimens captured that day belonged to *Lionepha chintimini* proved much more difficult than expected, in part because this group of beetles has only subtle morphological distinctions between species, and the first author at the time was inexperienced with the group. The effort, and its many twists and turns, combined with a puzzling specimen of a larger *Lionepha* found on the same day farther down the mountain, was the starting point of a long research journey. In the end, it was determined that *Lionepha chintimini* was indeed found that day, but that the species had previously been found in numbers by earlier collectors, and that it was a much more widespread species with an older name; the larger, mysterious specimen proved to be an elegant new species. The re-examination of the genus *Lionepha* thus inspired has culminated a decade later in the current work.

Lionepha are small, dark beetles (Figs 1–3) found in western North America from southern California to Alaska, east to Colorado. They can be abundant on the ground along the shores of creeks and lakes, as well as in open areas with damp soil, far from water (Fig. 4), from sea level to 3400 m in elevation.

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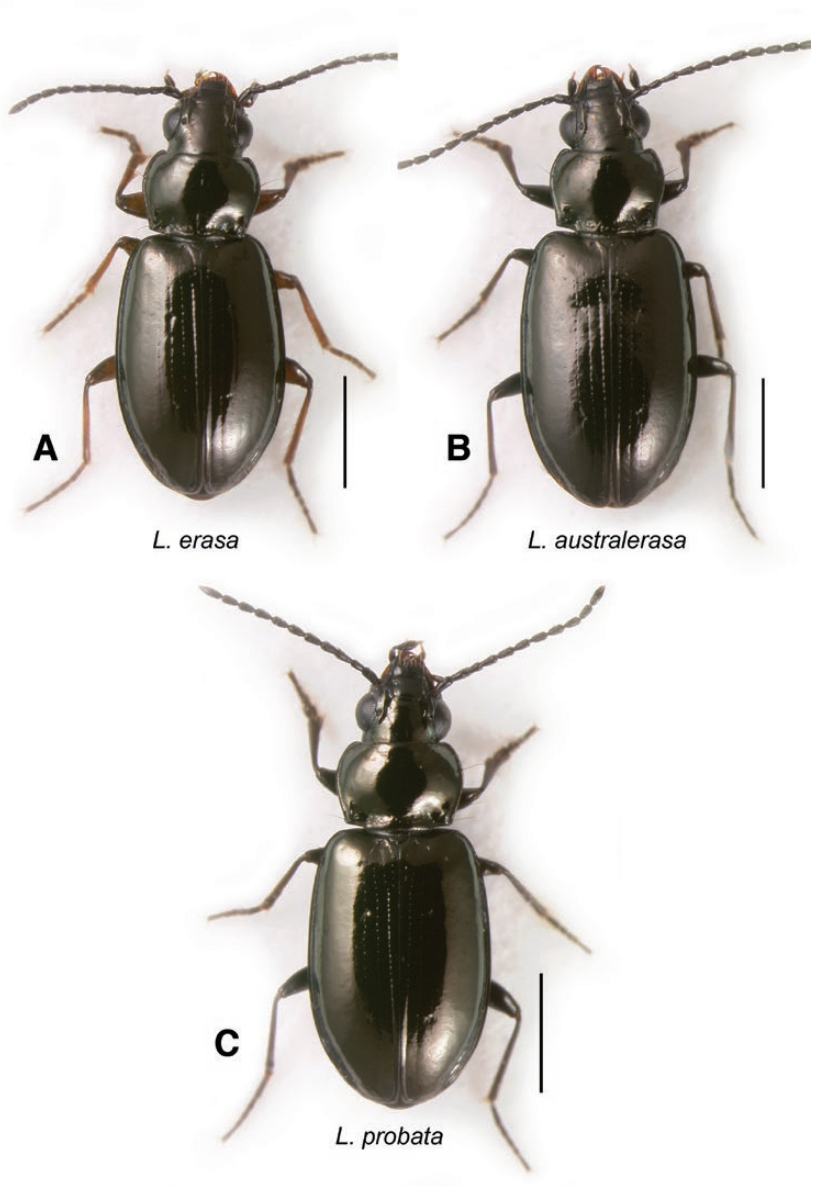

Figure 1. Adults of three species of the *Lionepha erasa* species group. A, *L. erasa*, USA: Oregon: Benton Co., Marys Peak; B, *L. australerasa*, holotype, USA: California: Amador Co., Oyster Lake, Silver Lake Campground; C, *L. probata,* USA: Oregon: Klamath Co., Odell Creek near Davis Lake. Images copyright David R. Maddison 2019, released under a Creative Commons CC-BY 4.0 license. Scale bar 1 mm.

There has been relatively little published on these beetles since the description of *Bembidium erasum* by LeConte (1859). Almost 60 years later, Casey (1918) described what we now recognize as three additional species, although he gave each of them two or three names. Lindroth's (1963) great work brought order to the group, and described three new species. His was also the first work to document male genitalic structures, habitats and geographic distributions of the species. The most recent work, Erwin & Kavanaugh's (1981) revision, was the first to hypothesize phylogenetic and biogeographic patterns within the group. In all of these works, the group was considered to belong within the large genus *Bembidion*; Maddison (2012) subsequently moved them to a separate genus, *Lionepha*, based upon evidence from DNA sequences.

Although the few studies of *Lionepha,* mostly limited to traditional topics in systematics, are typical for small, obscure beetles, a recent increase in research makes *Lionepha* a more compelling group

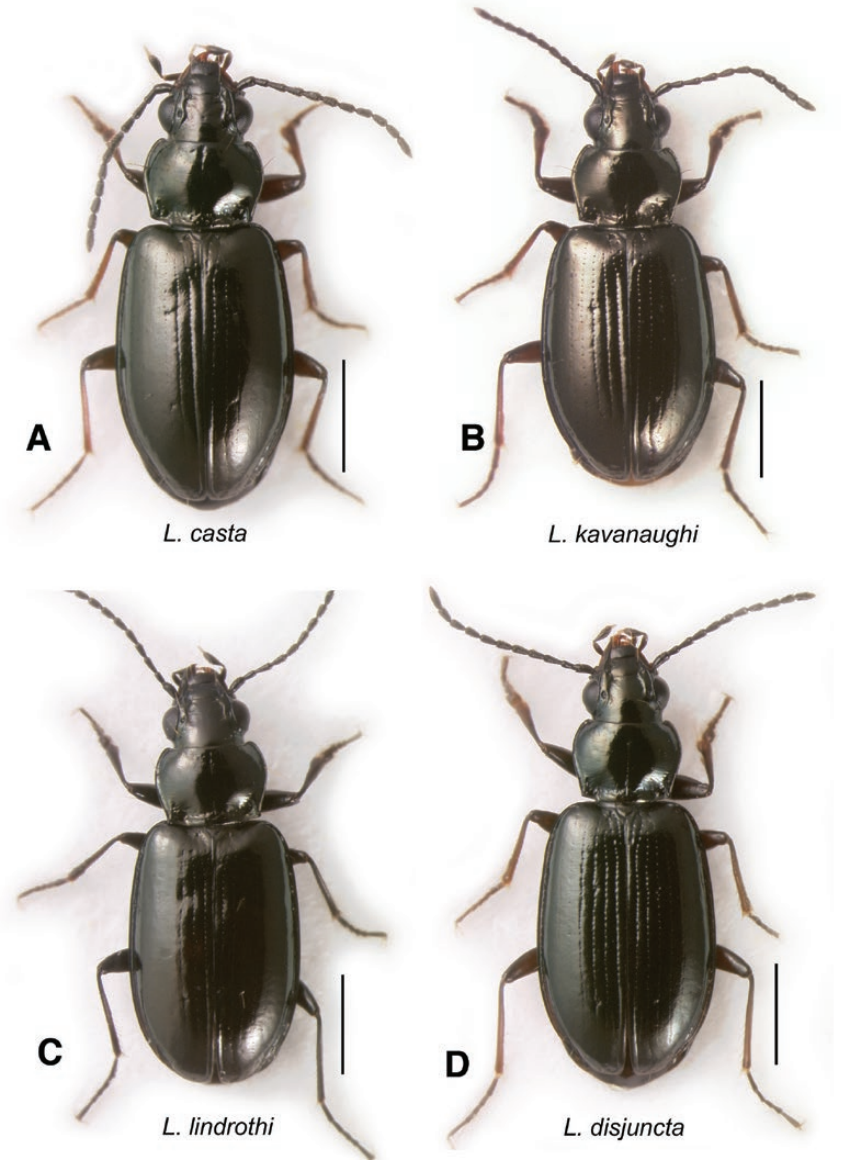

Figure 2. Adults of four species of the *Lionepha erasa* species group. A, *L. casta*, USA: Oregon: Benton Co., Marys Peak, Alder Creek Falls; B, *L. kavanaughi*, holotype, USA: Oregon: Wallowa Co., Lostine River Valley; B, *L. lindrothi*, holotype, USA: California: Tulare Co., outlet of Emerald Lake; D, *L. disjuncta,* Canada: British Columbia: Summit Creek 28 km E Kootenay Pass. Images copyright David R. Maddison 2019, released under a Creative Commons CC-BY 4.0 license. Scale bar 1 mm.

to clarify taxonomically. They have been used as a test case for sequencing old, pinned insects housed in museums (Sproul & Maddison, 2017), an effort that proved critical for the current study: settling on the names to be used for the species required sequencing of type specimens, including a LeConte lectotype collected between 1853 and 1857. The larger species found on Marys Peak, a new species here given the name *Lionepha tuulukwa*, has been chosen as a model for genomic studies within carabids, and has low-coverage genomic data available (Sproul & Maddison, 2017; Pflug *et al.*, in review), as well as measurements of its genome size and chromosomes (Pflug *et al*., in review).

In order to provide the basic systematic context for future studies of *Lionepha*, we here review the genus, using DNA sequence data combined with morphological evidence to delimit species within the group. We recognize 11 species of *Lionepha*, of which four are new. We provide tools to aid in identification of

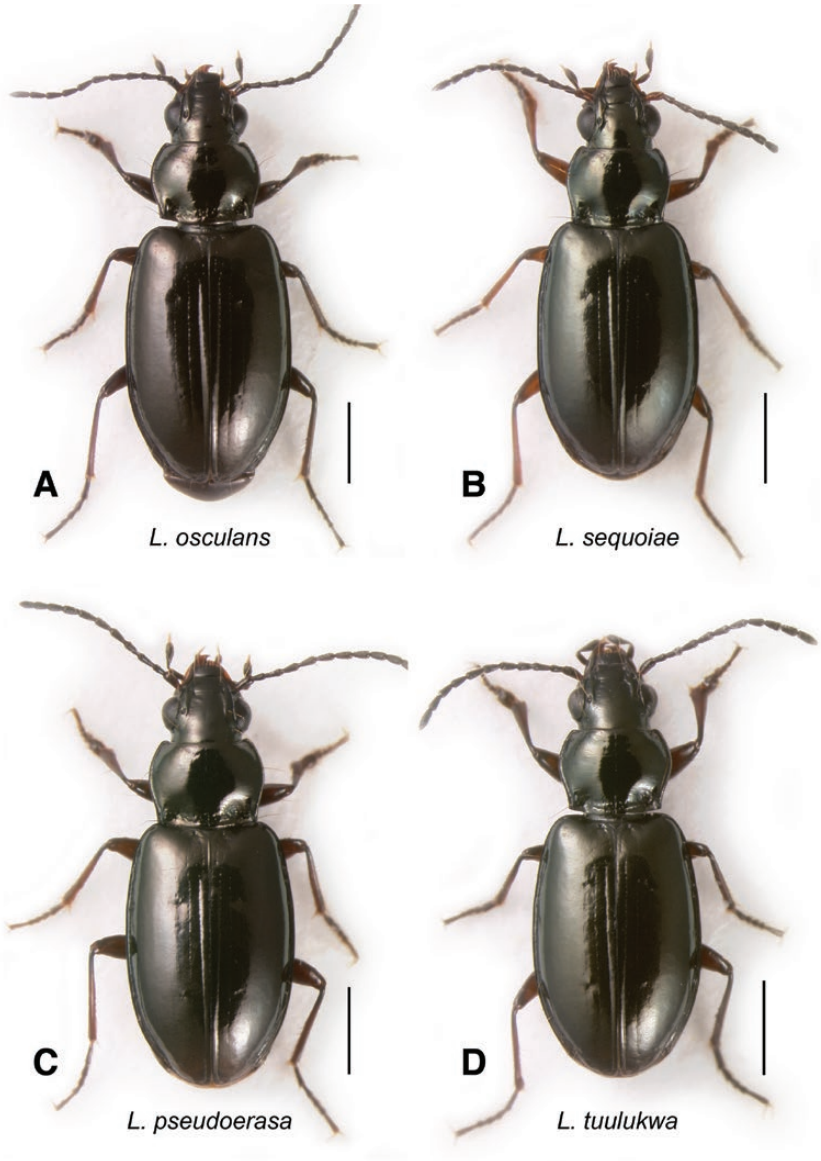

Figure 3. Adults of the *Lionepha osculans* species group. A, *L. osculans*, USA: California: Tehama Co., Nanny Creek; B, *L. sequoiae*, USA: Oregon: Lane Co., School Creek near Lookout Point Reservoir; C, *L. pseudoerasa*, USA: California: El Dorado Co., Strawberry Creek at Sciots Camp; D, *L. tuulukwa*, USA: Oregon: Benton Co., Marys Peak Rd, nr Alder Creek Falls. Images copyright David R. Maddison 2019, released under a Creative Commons CC-BY 4.0 license. Scale bar 1 mm.

specimens, as well as distribution maps of the known species.

MATERIAL AND METHODS

Approximately 2200 specimens of *Lionepha* were examined from the collections listed below; each collection listing begins with the code used in the text.

CAS	California Academy of Sciences, San Francisco, USA
CMNH	Carnegie Museum of Natural History, Pittsburgh, USA
CNC	Canadian National Collection of Insects, Ottawa, Canada
EMEC	Essig Museum Entomology Collection, University of California, Berkeley, USA
JRLC	James R. LaBonte Collection, Dallas, Oregon, USA

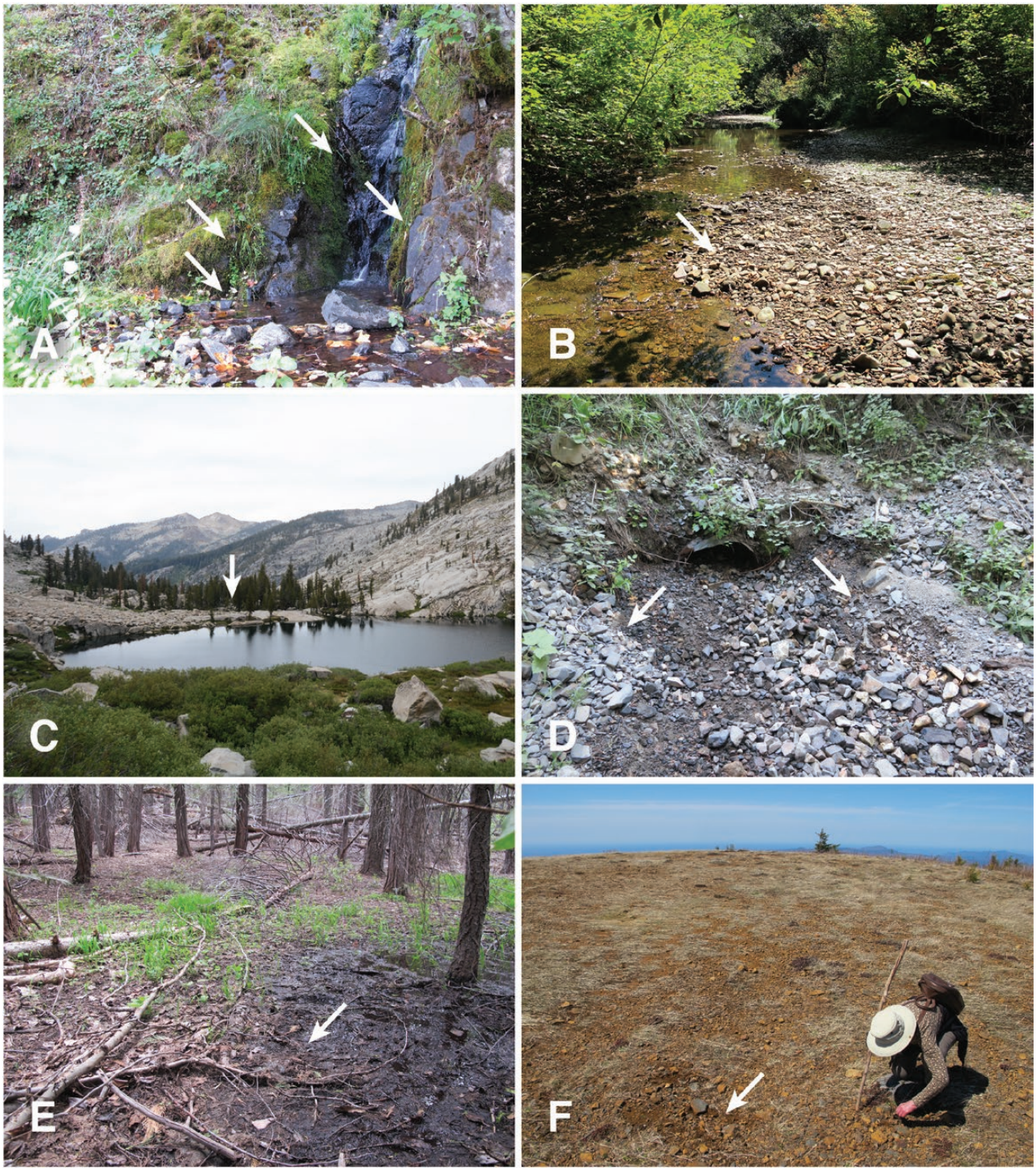

Figure 4. Habitats of *Lionepha*. Arrows indicate a typical microhabitat. A, habitat of *L. tuulukwa* and *L. casta*. USA: Oregon: Benton Co., Marys Peak Road, Alder Creek Falls, 700m, 44.4746°N 123.5286°W, 24 September 2010. B, habitat of *L. tuulukwa* and *L. casta*. *Lionepha osculans* has been found a few kilometres upstream along this same creek. USA: Oregon: Lane Co., Knowles Creek, 6.8 km SE Mapleton, 44.0136°N 123.7880°W, 9 August 2013. C, Environment of *L. lindrothi*. The beetles were found on dark organic shorelines of a partly shaded outlet stream of this lake, approximately at the point indicated by the arrow. USA: California: Tulare Co., outlet of Emerald Lake, 2814 m, 36.599°N 118.677°W, 21 June 2014.

CTVR Luca Toledano Collection, Verona, Italy
MCZ Museum of Comparative Zoology, Harvard University, Cambridge, UK
MNHN Muséum National d'Histoire Naturelle, Paris, France
MSBA Museum of Southwestern Biology, Division of Arthropods, University of New Mexico, Albuquerque, USA
MZLU Zoological Museum, Lund University, Lund, Sweden
NHMUK The Natural History Museum, London, UK
OSAC Oregon State Arthropod Collection, Oregon State University, Corvallis, USA
UAM University of Alaska Museum, University of Alaska, Fairbanks, USA
UASM University of Alberta Strickland Museum, Edmonton, Canada
UBC-SEM Spencer Entomological Collection of the Beaty Biodiversity Museum, University of British Columbia, Vancouver, Canada
USNM National Museum of Natural History, Smithsonian Institution, Washington, DC, USA
WSU M. T. James Museum, Washington State University, Pullman, USA

Collecting and storage methods

Specimens were collected by hand or by using an aspirator. During daylight, specimens were found in their habitats by removing surface layers of gravel, leaves or moss, or after splashing the soil with water, or after treading the soil and waiting for the beetles thus disturbed to appear on the surface. However, specimens were found more easily by collecting at night while wearing a headlamp, because at that time they are walking on the surface; night-time collecting is especially fruitful for those species not generally associated with bodies of water [e.g. *Lionepha erasa* (LeConte, 1859)].

Specimens for morphological studies were killed and preserved in *Acer* sawdust to which ethyl acetate was added. Specimens collected specifically for DNA sequencing were killed and stored in 95% or 100% ethanol, with best results obtained when the abdomen was slightly separated from the rest of the body to allow better penetration, or when the reproductive system was dissected out through the rear of the abdomen within a few seconds of the beetle's death in ethanol. Ethanol was decanted from vials and refilled at least once within the first few weeks after death. Storage was then at –20 °C. We do not know which methods were used to kill the dried specimens, collected before 1990, whose DNA was sequenced.

Morphological methods

Basic methods for studying adult structures, and terms used, are given in Maddison (1993). Genitalia, when studied, have been mounted in Euparal between two small coverslips attached to archival-quality heavyweight watercolour paper.

Photographs of entire beetles were taken with a Leica Z6Apo lens and DMC4500 camera, and of male genitalia with a Leica DM5500B compound microscope and DMC425C camera, with Leica Application Suite v.4.9 software capturing each image. Microsculpture photographs were taken with a DMC425C camera attached to a DM5500B compound scope equipped with an X-Cite 110LED light source, which provides co-axial illumination, and a 20× epi-illumination objective lens. For all photographs, a stack of images from different focal positions was merged using the PMax procedure in Zerene Systems's Zerene Stacker; the final images thus potentially have some artefacts caused by the merging algorithm.

Measurements were made using Leica Application Suite v.4.9 on the same imaging systems mentioned above. Body length measurements are the 'apparent body length', measured from the front of the labrum to the apex of the longest elytron. For some species, we also give the ratio of the width of the pronotum at its widest point divided by the length of the elytra (measured from the posterior edge of the scutellum to the tip of the longest elytron), abbreviated 'PW/EL'.

Terms used for structures within the internal sac of male genitalia of bembidiines have not been standardized across the tribe, with multiple divergent naming systems in use (e.g. Erwin & Kavanaugh, 1981; Maddison, 1993; Coulon, 2002). For the one sclerite we refer to by name, we follow Erwin & Kavanaugh's (1981) usage in order to be consistent with the most recent previous revision of *Lionepha*.

D, type locality of *L. kavanaughi*, as well as the habitat of *L. disjuncta* and *L. probata*, USA: Oregon: Wallowa Co., Lostine River Valley, 1483 m 45.3485°N 117.4152°W, 28 July 2016. The dry rocks in the centre of the channel were (before collecting began) along the edges of the channel. This channel, without flowing water, had a shallow, narrow flow of water two days earlier. E, habitat of *L. osculans, L. sequoiae, L. probata* and *L. australerasa*. USA: California: Tehama Co., Nanny Creek, Lassen NF, 1585 m, 40.3696°N 121.5607°W, 26 May 2013. F, habitat of *L. erasa*. USA: Oregon: Yamhill Co., Mount Hebo, 965 m, 45.2163°N 123.7583°W, 7 May 2012.

Elevational data

Elevational profiles of species were calculated by using the conversion tool in GPS Visualizer (Schneider, 2019) to estimate elevation for each georeferenced locality using standard digital elevation models. The lowest and highest elevations for each species were then verified by examining the records individually.

Cytogenetic methods

Fifteen *Lionepha* specimens were examined for chromosome number and sex-chromosome system. Methods used were as outlined by Maddison (1985, 2008).

Taxon sampling for DNA studies

We obtained sequence data from 147 specimens of all known species of *Lionepha* (Table 1), with between six and 35 specimens sampled per species, from as diverse geographic localities as possible. In addition, other species of Bembidiini, chosen to span the basal splits of related genera, were included as outgroups (Table 2), using data from previous publications (Maddison *et al.*, 1999; Maddison, 2008; Wild & Maddison, 2008; Maddison, 2012; Kanda *et al.*, 2015; Maddison & Anderson, 2016; Sproul & Maddison, 2017; Maddison *et al.*, 2019). Localities for the sequenced *Lionepha* specimens are given in Supporting Information, Table S1. Vouchers are deposited in OSAC, except for those specimens listed in Supporting Information, Table S2.

Included among these are six dried specimens that had been housed pointed or carded on pins in various museums for 58–159 years before DNA extraction. Some details about these specimens and their processing are provided in earlier publications. *Lionepha erasa* specimen 4002 has been reported on by Kanda *et al.* (2015), as *L. chintimini*. Sproul & Maddison (2017) reported on sequencing of the lectotype of *Bembidion erasum* (specimen 4241), the lectotype of *Bembidion brumale* Casey, 1918 (specimen 4893), a paralectotype of *Bembidion probatum* Casey, 1918 (specimen 4938), the holotype of *Bembidion lindrothellus* Erwin & Kavanaugh, 1981 (specimen 4891) and a specimen of *Lionepha casta* Casey, 1918 from Ketchikan, Alaska (specimen 4894).

DNA Sequencing

Genes studied and abbreviations used in this paper, are: **28S:** 28S ribosomal DNA (D1–D3 domains); **18S**: 18S ribosomal DNA (near full-length); ***COI***: cytochrome c oxidase I; ***wg***: *wingless*; ***CAD***: carbamoyl phosphate synthetase domain of the *rudimentary* gene; ***ArgK***: arginine kinase; ***Topo***: topoisomerase I; ***MSP***: Muscle Specific Protein 300.

For specimens collected into 95–100% ethanol (all specimens except numbers 4002, 4241, 4891, 4893, 4894 and 4938), DNA was extracted using a Qiagen DNeasy Blood and Tissue Kit. Fragments for the seven genes were amplified using the Polymerase Chain Reaction on an Eppendorf Mastercycler ProS Thermal Cycler, using TaKaRa Ex Taq and the basic protocols recommended by the manufacturers. Primers and details of the cycling reactions used are given in Maddison (2012) and Maddison & Cooper (2014). The amplified products were then cleaned, quantified and sequenced at the Genomic and Technology Core Facility of the University of Arizona using a 3730 XL Applied Biosystems automatic sequencer. Assembly of multiple chromatograms for each gene fragment and initial base calls were made with Phred (Green & Ewing, 2002) and Phrap (Green, 1999), as orchestrated by Mesquite's Chromaseq package (Maddison & Maddison, 2018a, 2018c) with subsequent modifications by Chromaseq and manual inspection. Multiple peaks at a single position in multiple reads were coded using IUPAC ambiguity codes.

Details about DNA extraction and sequencing of the six dried specimens were provided in Kanda *et al.* (2015, specimen 4002) and Sproul & Maddison (2017, specimens 4241, 4891, 4893, 4894 and 4938). DNA extraction and sequencing of dried specimens follows the protocols of those papers. In brief, DNA in specimen 4002 was extracted using the Qiagen DNeasy Blood & Tissue Kit, with a single-index library using the Apollo 324 NGS Prep System with the PrepX ILM DNA Library Kit (Wafergen), which was then sequenced on an Illumina HiSeq 2000, multiplexed on a 100-base paired-end lane. Reads for this specimen are archived on NCBI's Sequence Read Archive under accession number SRR2939021. The remaining five specimens were extracted using the Qiagen QIAmp Micro Kit (using the standard protocol with RNA carrier added), with dual-index libraries prepared using the NEBNext DNA Ultra II kit (New England BioLabs), which were then sequenced on an Illumina HiSeq 3000, multiplexed on either a 100-base or 150-base paired-end run. Illumina sequencing yielded 49 million to 141 million reads per specimen for these last five specimens (Table 3). Reads for these five specimens are archived on NCBI's Sequence Read Archive under the accession numbers given in Table 3.

Sequences for *Lionepha erasa* 4002 were obtained from the IlluminaMerged sequences of Kanda *et al.* (2015) with two exceptions. Although the reported *ArgK* (GenBank accession KU234030) matches that of a *Lionepha* for the first 310 bases, the last 101 bases do not, differing at ten amino acids for which all other *Lionepha* are constant. The last 101 bases were from a separate contig, and although the contig was not rejected by the protocol used in Kanda *et al.* (2015), we

Table 1. Examined DNA sequences of *Lionepha* specimens. Four-digit numbers in the '#' column are D. R. Maddison DNA voucher numbers. The four specimens whose numbers followed by * (3844 under *L. australerasa*, 4117 under *L. lindrothi*, 5000 under *L. kavanaughi* and 2643 under *L. tuulukwa*) are holotypes of the new species. Sequences newly acquired are shown as check marks; those with GenBank accession numbers were previously published

	#	28S	*COI*	*CAD*	*Topo*	*ArgK*	*MSP*	*wg*	18S
Lionepha erasa									
BC: Cherryville	4002	KU233792	*KU233842*	KU233980	KU234077	KU234030	✓	*KU233867*	KU233692
AK: Thompson Pass	4059	KU233784	*KU233833*	KU233943	KU234069	KU233993	✓	*KU233880*	KY246689
AK (*B. lindrothellus* holotype)	4891	✓	✓	✓	✓	✓	✓	✓	✓
OR (*B. erasum* lectotype)	4241	✓	✓	✓			✓		✓
OR: Marys Peak	2575	✓	✓	✓	✓				
OR: Prairie Peak	2580	✓	✓	✓	✓	✓		✓	
OR: Marys Peak	2586	✓	✓	✓	✓				
OR: Marys Peak	2615	✓	✓	✓	✓	✓		✓	
OR: Marys Peak	2616	KY246706	*KY246747*	KY246787	KY246828	✓	✓	✓	KY246675
OR: Mount Hebo	3013	✓	✓	✓	✓			✓	
OR: Mount Hebo	3016	✓	✓	✓	✓			✓	
OR: Mount Hood	4144	KY246724	*KY246765*	KY246805	KY246846	✓	✓	✓	KY246690
OR: Lost Prairie	5197	✓	✓	✓	✓				
OR: Lost Prairie	5199	✓	✓	✓	✓		✓	✓	
OR: Lost Prairie	5200	✓	✓	✓	✓				
OR: Lost Prairie	5201	✓	✓	✓	✓				
Lionepha australerasa									
CA: Martin Meadow	3838	✓	✓	✓	✓				
CA: Carson Spur	3839	✓	✓	✓	✓				
CA: Carson Spur	3840	✓	✓	✓	✓				
CA: Carson Spur	3841	✓	✓	✓	✓		✓		
CA: Oyster Lake	3844*	KY246720	*KY246761*	KY246801	KY246842	✓	✓	✓	KY246686
CA: Oyster Lake	3845	✓	✓	✓	✓				
CA: Nanny Creek	3864	KY246721	*KY246762*	KY246802	KY246843	✓	✓	✓	KY246687
CA: Nanny Creek	3896	✓	✓	✓	✓				
CA: Mill Creek	5212	✓	✓	✓	✓			✓	
CA: Mill Creek	5213	✓	✓	✓	✓			✓	
CA: Homewood Canyon	5214	✓	✓	✓	✓		✓		
OR: Munson Creek	4984	✓	✓	✓	✓		✓		
OR: Munson Creek	4986	✓	✓	✓	✓		✓	✓	
Lionepha probata									
CA: Middle Martis Creek	1161	✓	✓		✓	JN170707	✓	*JN171546*	
WA: Taneum Creek	1320	JN170469	*JN171142*	JN170949	JN171321				JN170253
CA: Middle Martis Creek	1970	✓	✓	✓	✓	✓	MK121268	✓	
OR: Steens Mountains	2724	✓	✓	✓	✓				
OR: Mount Ashland	3165	✓	✓	✓	✓				
UT: Stansbury Mountains	3601	✓	✓	✓	✓	✓	✓	✓	

Table 1. Continued

	#	28S	*COI*	*CAD*	*Topo*	*ArgK*	*MSP*	*wg*	18S
NV: Ruby Mountains	3684	✓	✓	✓	✓			✓	
UT: Stansbury Mountins	3685	✓	✓	✓	✓			✓	
CA: Bishop Creek	3686	✓	✓	✓	✓			✓	
CA: Steens Mountains	3717	✓	✓	✓	✓				
BC: Summit Creek	3720	✓	✓	✓	✓		✓		
ID: Galena Summit	3722	✓	✓	✓	✓				
OR: Lost Prairie	3723	KY246717	*KY246758*	KY246798	KY246839				KY246683
CA: Sherman Pass	3730	✓	✓	✓	✓				
CA: Strawberry Creek	3832	✓	✓	✓	✓				
CA: White Mountains	3833	✓	✓	✓	✓		✓	✓	
WA: Blue Mountains	3854	✓	✓	✓	✓				
CA: Algoma Camp	3855	✓	✓	✓	✓				
CA: Warner Range	3863	✓	✓	✓	✓				
ID: Baker Creek	3865	✓	✓	✓	✓				
ID: Park Creek	3866	✓	✓	✓	✓				
OR: Odell Creek	3867	KY246722	*KY246763*	KY246803	KY246844				
CA: Nanny Creek	3895	✓	✓	✓	✓				
CA: Deadman Creek	4137	✓	✓	✓	✓	✓		✓	
CA: Ellery Lake	4138	✓	✓	✓	✓	✓		✓	
UT: Shingle Creek	4198	✓	✓	✓	✓			✓	
MT: Thompson Pass	4645	✓	✓	✓	✓		✓		
MT: Mill Creek	4713	✓	✓	✓	✓				
OR: Ochoco NF	4744	KY246739	*KY246780*	KY246820	KY246861				
CO (*B. probatum* paralectotype)	4938	✓	✓		✓		✓		✓
OR: Lostine River Valley	4991	✓	✓	✓	✓	✓	✓	✓	
OR: Little Phillips Creek	4995	✓	✓	✓	✓				
OR: Lostine River	5004	✓	✓	✓	✓				
UT: Tushar Mountains	5037	✓	✓	✓	✓		✓		
CA: Squaw Valley	5211	✓	✓	✓	✓				
Lionepha casta									
WA: Taneum Creek	1400	JN170467	*JN171140*	JN170947	JN171319	JN170705	✓	*JN171544*	
OR: Marys Peak	2545	KY246705	*KY246746*	KY246786	KY246827	✓	✓	✓	KY246674
OR: School Creek	3041	✓	✓	✓	✓		✓		
OR: Lost Prairie	5204								
CA: West Branch Mill Creek	3703	✓	✓	✓	✓	✓		✓	
CA: Mt. Tamalpais	3830	✓	✓	✓	✓		✓		
CA: Soda Creek	4049	✓	✓	✓	✓				
AK: Prince of Wales Island	4523	KY246736	*KY246777*	KY246817	KY246858	✓		✓	KY246699
BC (*B. brumale* lectotype)	4893	✓	✓						✓
AK: Ketchikan	4894	✓	✓		✓		✓	✓	✓
Lionepha kavanaughi									
MT: Nez Perce Pass	4646	✓	✓	✓	✓	✓	✓	✓	✓

Table 1. Continued

	#	28S	*COI*	*CAD*	*Topo*	*ArgK*	*MSP*	*wg*	18S
MT: Lost Horse Creek	4648	KY246738	*KY246779*	KY246819	KY246860	✓	✓	✓	
OR: Lostine River Valley	4990	✓	✓	✓	✓				
OR: Lostine River Valley	4992	✓	✓	✓	✓	✓	✓	✓	
OR: Lostine River Valley	4993	✓	✓	✓	✓		✓	✓	
OR: Lostine River Valley	4996	✓	✓	✓	✓				
OR: Little Phillips Creek	4998	✓	✓	✓	✓	✓	✓	✓	✓
OR: Lostine River Valley	5000*	✓	✓	✓	✓	✓	✓	✓	✓
OR: Lostine River Valley	5002	✓	✓	✓	✓				
OR: Lostine River Valley	5006	✓	✓	✓	✓		✓	✓	
OR: Lostine River Valley	5008	✓	✓	✓	✓				
OR: Lostine River Valley	5010	✓	✓	✓	✓		✓	✓	
Lionepha lindrothi									
CA: Bishop Creek	3568	KY246716	*KY246757*	KY246797	KY246838	✓	✓	✓	KY246682
CA: Emerald Lake	4116	✓	✓	✓	✓	✓		✓	
CA: Emerald Lake	4117*	✓	✓	✓	✓	✓	✓	✓	
CA: Emerald Lake	4118	✓	✓	✓	✓	✓		✓	
CA: East Fork Kaweah River	4120	✓	✓	✓	✓			✓	
CA: Kaiser Pass	4121	✓	✓	✓	✓	✓		✓	
CA: Tioga Lake	4132	✓	✓	✓	✓	✓		✓	
CA: Sonora Pass	4134	✓	✓	✓	✓	✓		✓	
CA: Deadman Creek	4140	✓	✓	✓	✓	✓		✓	
CA: San Jacinto Mountains	5072	✓	✓	✓	✓		✓	✓	
Lionepha disjuncta									
BC: Summit Creek	1896	JN170468	*JN171141*	JN170948	JN171320	JN170706	✓	*JN171545*	JN170252
CA: Lily Lake	3069	✓	✓	✓	✓	✓	✓	✓	
BC: Summit Creek	3090	✓	✓	✓	✓				
OR: Lostine River Valley	3848	✓	✓	✓	✓				
CA: Trinity Alps	4115	✓	✓	✓	✓	✓		✓	
CA: Emerson Creek	4122	✓	✓	✓	✓	✓		✓	
CA: Salmon Creek	4133	✓	✓	✓	✓	✓		✓	
OR: Mount Hood	4143	✓	✓	✓	✓	✓	✓	✓	
MT: Mill Creek	4716	✓	✓	✓	✓				
ID: North Fork Salmon River	4780	✓	✓	✓	✓				
Lionepha osculans									
CA: Cold Creek	1387	✓	✓	✓	✓			✓	
CA: Cold Creek	1390	✓	✓		✓				
CA: Rainbow	1401	JN170470	*JN171143*	JN170950	JN171322	JN170708		*JN171547*	JN170254
OR: School Creek	2638	✓	✓	✓	✓	✓	MK121302	✓	
OR: Berry Creek	3095	✓	✓	✓	✓				
CA: Sanislaus NF	3157	✓	✓	✓	✓				
OR: Goodman Creek	3158	✓	✓	✓	✓				

Table 1. Continued

	#	28S	*COI*	*CAD*	*Topo*	*ArgK*	*MSP*	*wg*	18S
CA: Warner Range	3161	✓	✓	✓	✓				
CA: Los Padres NF	3162	✓	✓	✓	✓				
CA: Strawberry Creek	3163	✓	✓	✓	✓				
CA: Carson Spur	3164	✓	✓	✓	✓				
CA: Nanny Creek	3721	✓	✓	✓	✓				
CA: Pike County Gulch	3846	✓	✓	✓	✓	✓		✓	
OR: Eugene	4593	✓	✓	✓	✓		✓		
OR: Walton Lake	4743	✓	✓	✓	✓				
OR: Little Phillips Creek	5001	✓	✓	✓	✓				
Lionepha sequoiae									
OR: School Creek	2614	✓	✓	✓	✓	✓	✓	✓	✓
OR: Oakridge	2647	✓	✓	✓	✓				
CA: Strawberry Creek	3075	✓	✓	✓	✓	✓	✓	✓	
CA: Bridal Veil Falls	3078	✓	✓	✓	✓				
CA: Strawberry Creek	3085	✓	✓	✓	✓				
CA: Nanny Creek	3702	✓	✓	✓	✓				
Lionepha pseudoerasa									
CA: Strawberry Creek	3072	✓	✓	✓	✓	✓	✓	✓	✓
CA: Lily Lake	3073	✓	✓	✓	✓	✓		✓	
CA: Strawberry Creek	3083	✓	✓	✓	✓				
CA: Strawberry Creek	3086	✓	✓	✓	✓				
CA: Strawberry Creek	3087	✓	✓	✓	✓				
CA: Sherman Pass	3599	✓	✓	✓	✓	✓		✓	
CA: Sherman Pass	3688	✓	✓	✓	✓	✓		✓	
CA: Trinity Alps	4114	✓	✓	✓	✓	✓	✓	✓	
CA: Kaiser Pass	4139	✓	✓	✓	✓	✓		✓	
Lionepha tuulukwa									
OR: Marys Peak	2581	✓	✓	✓	✓				
OR: Marys Peak	2635	✓	✓	✓	✓				
OR: Marys Peak	2636	✓	✓	✓	✓				
OR: Marys Peak	2637	✓	✓	✓	✓				
OR: Marys Peak	2642	✓	✓	✓	✓	✓		✓	
OR: Marys Peak	2643*	✓	✓	✓	✓	✓	✓	✓	✓
OR: Knowles Creek	3700	✓	✓	✓	✓	MK838505	✓	*MK838494*	
OR: Knowles Creek	3701	✓	✓	✓	✓				
OR: Marys Peak	3782	KY246718	*KY246759*	KY246799	KY246840				KY246684
CA: Trinity Alps	4113	✓	✓	✓	✓	✓	✓	✓	

Table 2. Outgroup taxa and GenBank accession numbers of sequences used

	28S	*COI*	*CAD*	*Topo*	*ArgK*	*MSP*	*wg*	18S
Sinechostictus (Pseudolimnaeum) sp. 3	JN170474	JN171150	JN170960	JN171329	JN170717	MK121236	JN171552	JN170259
Sinechostictus elongatus (Dejean, 1831)	JN170479	JN171152	JN170965	JN171332	JN170722	MK121235	JN171557	JN170260
Ocys harpaloides (Audinet-Serville, 1821)	KX907154	KX907141	KX907179	KX907188	KX907173	MK121306	KX907170	KX907168
Ocys quinquestriatus (Gyllenhal, 1810)	JN170472	JN171145	JN170954	JN171324	JN170712		JN171549	JN170257
Amerizus wingatei (Bland, 1864)	JN170267	JN170974	JN170732	JN171160	JN170485	MK121246	JN171339	JN170136
Amerizus (Tiruka) sp.	JN170265	JN170972	JN170730	JN171158	JN170483	MK121273	JN171337	JN170134
Asaphidion yukonense Wickham, 1919	JN170273	JN170979	EU677540	EU677638	EU677515	MK121263	EU677666	JN170139
Asaphidion curtum (Heyden, 1870)	GU556078	JN170977	JN170736	JN171163	JN170489	MK121210	GU556027	AF002792
Bembidion chalceum Dejean, 1831	EF648892	EF649200	EF649431	EU677650	EF648737	MK121245	EF649548	EF648647
Bembidion transversale Dejean, 1831	EU677688	GU454797	EU677541	EU677639	JN170691		EU677667	JN170242
Bembidion variegatum Say, 1823	JN170458	JN171131	JN170937	JN171310	JN170695	MK121242	JN171535	JN170245
Bembidion rapidum (LeConte, 1847)	EU677690	JN171095	EU677543	EU677642	EU677518		EU677668	JN170224

Table 3. Sequences obtained for pinned, dried specimens. Voucher numbers are given under '#'. Archive numbers refer to the accession numbers in NCBI's Sequence Read Archive, as published by Sproul & Maddison (2017). Numbers under each gene indicate the length in bases of the sequence obtained that included that gene. Reads are the approximate number of sequencing reads obtained, in millions

#	Archive	Reads	Assembly	28S	18S	COI	*CAD*	*Topo*	*ArgK*	*MSP*	*wg*
4241	SRR5230423	86	*de novo*	5827	5368	756	0	0	0	0	0
4241	SRR5230423	86	ref-based	-	-	-	312	39	0	125	72
4891	SRR5230400, SRR5230401	115	*de novo*	7260	6182	16437	789	280	361	762	275
4893	SRR5230408	49	*de novo*	3648	5120	4040	0	0	0	0	0
4938	SRR5230412	53	*de novo*	13571	13571	3141	0	257	0	310	0
4894	SRR5230402, SRR5230403	141	*de novo*	11771	11771	16351	0	250	0	728 + 274	375

exclude it, and those 101 bases, from consideration. *MSP* was not reported by Kanda *et al.* (2015); we acquired it by BLASTing the *MSP* of *Lionepha osculans* (Casey, 1918) DNA2638 against the *de novo* assembly of specimen 4002. This yielded two slightly overlapping contigs (which were identical in the region of overlap). These were merged and form the sequence reported here.

For the remaining five dried specimens, we generated sequences to be included in our analyses using the following protocol. Reads were processed in CLC Genomics Workbench v.8.5. We trimmed reads to eliminate low-quality ends (limit = 0.05) and to remove adapter sequences. *De novo* assemblies were generated using Genomics Workbench from paired, trimmed reads using an automatic word and bubble size, with the minimum contig length set to 200. The *de novo* assemblies were converted to BLASTable databases using NCBI's makeblastdb tool and BLASTed using Mesquite's (Maddison & Maddison, 2018c) local BLAST tool (1E-100 as the e-value cut-off, and up to 30 hits), using the sequences of *Lionepha tuulukwa* 2643 as query sequences. With two exceptions, this yielded zero or one contig per library per gene, ranging in size from 250 to 16 437 bases (Table 3). One exception was the type of *Bembidion brumale*, which had two 18S contigs, one 5120 bases in length, and which BLASTed to beetle

sequences in NCBI's GenBank, and the other 832 bases in length, which BLASTed to the fungus *Rhizopus*, and was discarded. In addition, specimen 4894 of *Lionepha casta* had two non-overlapping *MSP* contigs, both of which were kept as they both BLAST to beetles.

For the lectotype of *Bembidium erasum*, *de novo* assembly yielded no sequences for the nuclear protein-coding genes studied here. As a result, reference-based assembly, using *Lionepha tuulukwa* 2643 sequences as the reference, was conducted using CLC Genomics Workbench's read mapping feature. This yielded sequences for *CAD, Topo, MSP* and *wg*. However, for both *Topo* and *wg*, the sequences were short. The 39 bases recovered for *Topo* show no distinguishing bases between *L. australerasa*, *L. disjuncta* (Lindroth, 1963) and *L. erasa*; the 72 bases recovered for *wg* show no distinguishing bases between *L. australerasa, L. disjuncta*, *L. erasa* and the four members of the *L. osculans* species group. These short sequences were excluded from further consideration, as in the phylogenetic analyses they were likely to be placed randomly with any of these species.

Sequences have been deposited in GenBank with accession numbers MN401767 through MN402440. Alignments and trees are presented in a NEXUS file for use in Mesquite in Supporting Information, File S1, and are also deposited in the Dryad data repository (https://doi.org/10.5061/dryad.2jm63xsjq).

Sequence alignment

Alignment was not difficult for any of the protein-coding genes. There were no insertions or deletions (indels) evident in the sampled *CAD, ArgK, Topo* or *COI* sequences. In *wingless*there was one insertion of three nucleotides (corresponding to the addition of a single amino acid, asparagine, in the protein) in the three specimens of *Lionepha probata* (Casey, 1918) from Utah for which *wingless* was obtained (two from the Stansbury Mountains and one from the Uinta Mountains). An alignment of 28S and 18S was performed by MAFFT v.7.130b (Katoh & Standley, 2013), using the L-INS-i search option and otherwise default parameter values.

Sites in 28S and 18S were chosen to be excluded from consideration using the modified GBLOCKS analysis (Talavera & Castresana, 2007) present in Mesquite with the following options: minimum fraction of identical residues for a conserved position = 0.2, minimum fraction of identical residues for a highly-conserved position = 0.4, counting fraction within only those taxa that have non-gaps at that position, maximum number of contiguous non-conserved positions = 4, minimum length of a block = 4, and allowed fraction of gaps within a position = 0.5.

Molecular phylogenetic analysis

Maximum likelihood analysis was conducted for each gene individually using IQ-TREE v.1.6.7.1 (Nguyen *et al.*, 2015), as orchestrated by Mesquite's Zephyr package (Maddison & Maddison, 2018b, 2018c). The ModelFinder feature within IQ-TREE (Kalyaanamoorthy *et al.*, 2017) was used to find the optimal character evolution models. The MFP model option was used for 28S and 18S, and the TESTMERGE option for the protein-coding genes. The TESTMERGE option caused IQ-TREE to seek the optimal partition of sites, with the initial partition having the codon positions in different parts. One hundred searches were conducted for the maximum-likelihood tree for each matrix; for bootstrap analyses, 500 replicates were used. The maximum likelihood tree for a matrix with all eight genes concatenated was also sought, using 100 search replicates, also using the TESTMERGE option in which the starting partition had 20 parts (three codon positions for each of six protein-coding genes, and then the two ribosomal genes).

For the 141 *Lionepha* specimens plus 12 outgroup specimens for which we obtained all four primary genes (28S, *COI*, *CAD* and *Topo*) we used a multispecies coalescent approach to provide an algorithmic analysis of species boundaries. We used STACEY v.1.2.4 (Jones, 2017) as implemented in BEAST v.2.5.5 (Bouckaert *et al.*, 2014), with the epsilon value set to 1*10–4, CollapseWeight parameters to 0.5 and 10, and with a Beta prior. We evaluated sampling sufficiency using ESS values in TRACER v.1.7.1 (Rambaut *et al.*, 2018), and halted the analysis only after all ESS values exceeded 200. This occurred after four independent runs of 1E9 generations each. As we saved trees every 100 000 generations, with the first 10% of the trees discarded as the burn-in period, this yielded a sample of 72 000 trees.

RESULTS

Molecular phylogenetic results

Maximum likelihood trees (Figs 5–7) show some consistency from gene to gene, but also some notable differences. The larger *Lionepha* (*osculans* group) form a clade in seven of the eight genes, with bootstrap support ranging from 57 to 100 (Table 4), except in *ArgK*, for which the group forms a grade at the base of *Lionepha* (Fig. 7). The smaller *Lionepha* (*erasa* group) form a clade in four genes, with support ranging from 56 to 100 (Table 4). Not visible in the figures (as the outgroups were graphically removed) is support for monophyly of *Lionepha* as a whole. The genus is strongly supported as monophyletic, with bootstrap support in seven of the eight genes being 100, and

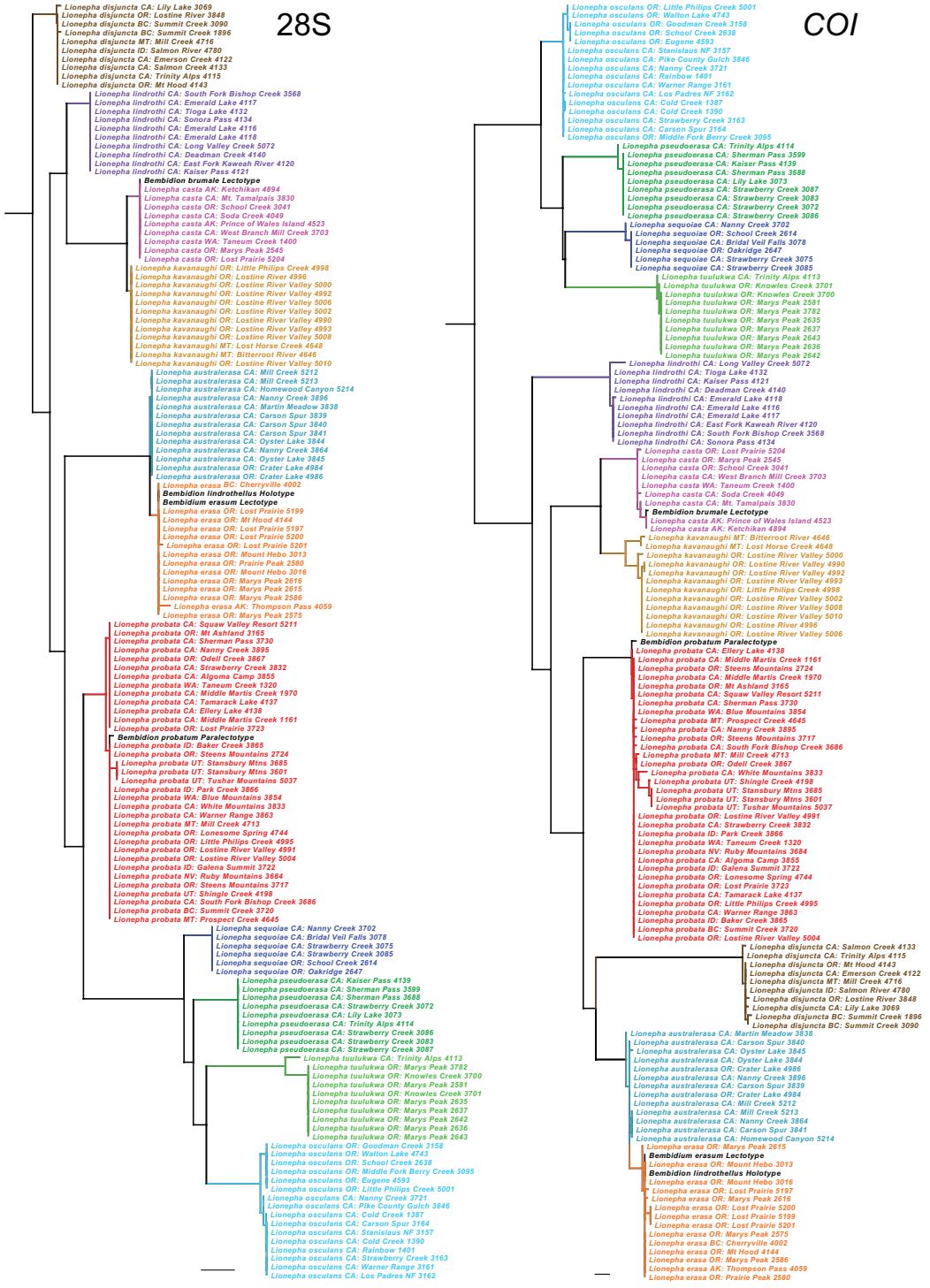

Figure 5. Maximum likelihood trees for 28S and *COI*. Branch length is shown proportional to relative divergence, as estimated by IQ-TREE; scale bars indicate 0.01 units. Outgroups not depicted.

the other gene (*COI*) showing bootstrap support of 90 (Table 4).

Within the *L. erasa* group, some species-pairs are supported as monophyletic, and some higher level structure is evident, although with one exception support is not universal across genes. The morphologically similar trio of *L. australerasa*, *L. erasa* and *L. probata* is supported as a clade by

Figure 6. Maximum likelihood trees for *CAD* and *Topo*. Branch length is shown proportional to relative divergence, as estimated by IQ-TREE; scale bars indicate 0.01 units. Outgroups not depicted.

four genes, with *L. erasa* + *L. australerasa* supported by all eight genes (Table 4). This trio appears to be related to *L. disjuncta*, a result strongly supported by *ArgK* and 18S, and weakly supported by two other genes. The other trio of structurally similar small *Lionepha*, *L. casta* + *L. kavanaughi* + *L. lindrothi*, is strongly supported as a clade by three genes, and weakly supported by two others, with *L. casta* and

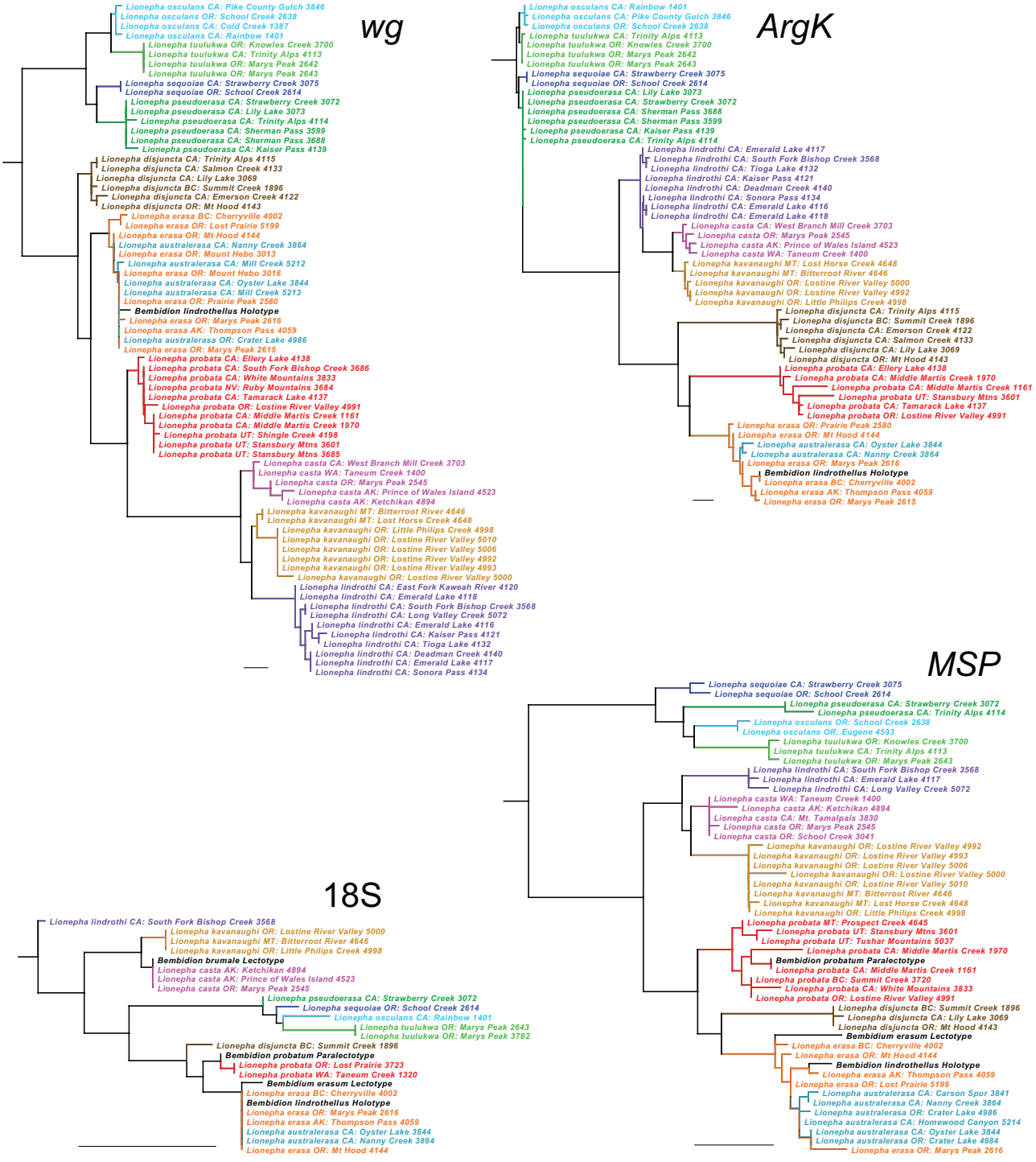

Figure 7. Maximum likelihood trees for *wg*, *ArgK*, 18S and *MSP*. Branch length is shown proportional to relative divergence, as estimated by IQ-TREE; scale bars indicate 0.01 units. Outgroups not depicted.

L. kavanaughi evidently being sister groups, a result consistent with their similar male and female genitalia.

The monophyly of the *L. osculans* group, the *L. erasa* group, *L. erasa* + *L. australerasa* + *L. probata*, and *L. casta* + *L. kavanaughi* + *L. lindrothi* are also supported by multigene analyses (STACEY Bayesian analysis, Fig. 8; maximum likelihood tree from a concatenated matrix, Supporting Information, Fig. S1). The multigene trees are also consistent with the phylogeny inferred by Erwin & Kavanaugh (1981).

Table 4. Support for and against various clades. One or two values are given in each cell. If the bootstrap support percentage is 90 or more, only that value is listed. If bootstrap support is less than 90, two values are listed: the bootstrap support for the clade, followed by a negative value, which is the bootstrap support against the clade, as measured by the bootstrap value for the contradictory clade with the highest support value. Colour of cells are as explained in the legend. '-' indicates that only a single specimen was sampled for that gene and thus the monophyly could not be tested. '#g' give the number of genes for which there is bootstrap support of 50 or more for the clade

	28S	*COI*	*CAD*	*Topo*	*wg*	*MSP*	*ArgK*	18S	#g
Lionepha	100	90	100	100	100	100	100	100	8
L. erasa group	2, -48	63, -13	1, -50	36, -55	56, -38	87, -5	100	3, -79	4
L. eras + aus + prob + disj	4, -48	49, -20	13, -50	34, -55	16, -44	72, -14	99	90	4
L. eras + aus + prob	27, -34	20, -24	51, -12	54, -22	1, -44	8, -50	24, -45	74, -16	3
L. erasa + australerasa	100	63, -16	74, -5	75, -6	73, -5	85, -6	89, -3	96	8
L. casta + kav + lind	27, -28	3, -38	31, -32	98	100	87, -4	48, -45	23, -68	3
L. casta + kavanaughi	100	67, -12	1, -32	25, -38	16, -45	65, -15	83, -11	93	5
L. osculans group	100	57, -23	99	83, -16	65, -16	66, -29	24, -63	100	7
L. erasa	94	62, -11	1, -29	0, -24	0, -48	0, -61	29, -28	-97	2
L. australerasa	45, -17	35, -36	0, -29	36, -18	0, -48	15, -51	-	-	0
L. probata	91	90	36, -11	79, -8	61, -16	96	100	-	6
L. casta	97	90	0, -48	65, -8	55, -30	83, -10	94	81, -18	7
L. kavanaughi	97	48, -25	84, -4	92	59, -28	97	84, -11	99	7
L. lindrothi	99	94	7, -13	99	92	97	23, -27	-	5
L. disjuncta	98	97	88, -6	92	66, -15	100	100	-	7
L. osculans	100	77, -6	88, -4	55, -16	88, -5	-	89, -5	-	6
L. sequoiae	99	88, -9	85, -3	91	85, -7	76, -14	95	-	7
L. pseudoerasa	99	96	59, -24	97	84, -7	99	18, -35	-	6
L. tuulukwa	100	98	13, -25	97	99	97	52, -25	-	6
Lionepha specimens sequenced	143	143	140	143	70	47	52	21	

Legend

Black cells: bootstrap support for the clade 90 or above
Gray cells: bootstrap support for the clade 50-89
White cells: ML tree has clade, with bootstrap support 0-49
Pink cells: ML tree has contradictory clade, with bootstrap support 0-49
Red cells: bootstrap support against clade 50-100

INDELS IN 28S

There are data in the DNA sequences not considered in any of the phylogenetic analyses, in the form of indels. The 28S gene, in particular, shows an amount of insertions and deletions in the evolutionary history of *Lionepha* that is unusual within bembidiines; every species of *Lionepha* has a unique set of indels. Many of these indels are in a part of the D2 expansion region (Supporting Information, Fig. S2). There is variation within species as well. *Lionepha disjuncta*, in particular, has striking variation: among the ten specimens sequenced there are eight unique patterns of insertions and deletions over three regions of the gene. There was less variation observed in other species: four bases missing from the northernmost specimen of *L. erasa* and an extra two bases in the Mt. Hood specimen; an extra two bases in two Oregon and one Washington specimens of *L. casta*; an extra base in the Utah specimens of *L. probata*; four indel differences between the California and Oregon specimens of *L. tuulukwa*.

MORPHOLOGICAL RESULTS

The morphological diversity evident among specimens of *Lionepha* (depicted in Figs 9–19, and described in

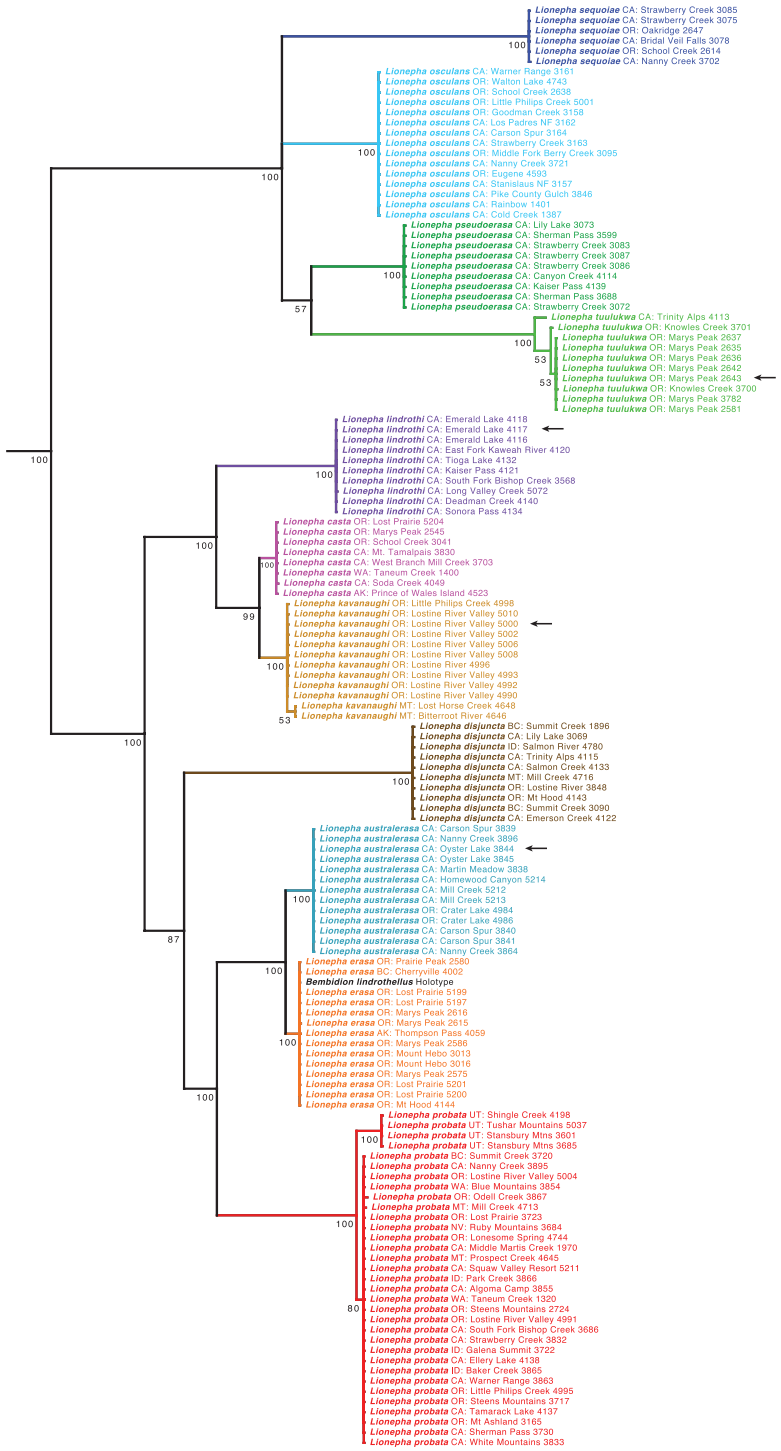

Figure 8. Majority rule consensus tree of trees found from a STACEY analysis. Numbers on branches are estimates of the Bayesian posterior probability of a clade, expressed as a percentage. Outgroups not depicted. The arrows indicate the holotypes of the new species.

'Taxonomic treatment', below) corresponds to the patterns evident in the gene trees, with the male genitalic structures in particular (Figs 11–14) being consistent within a proposed species and showing disparities among species.

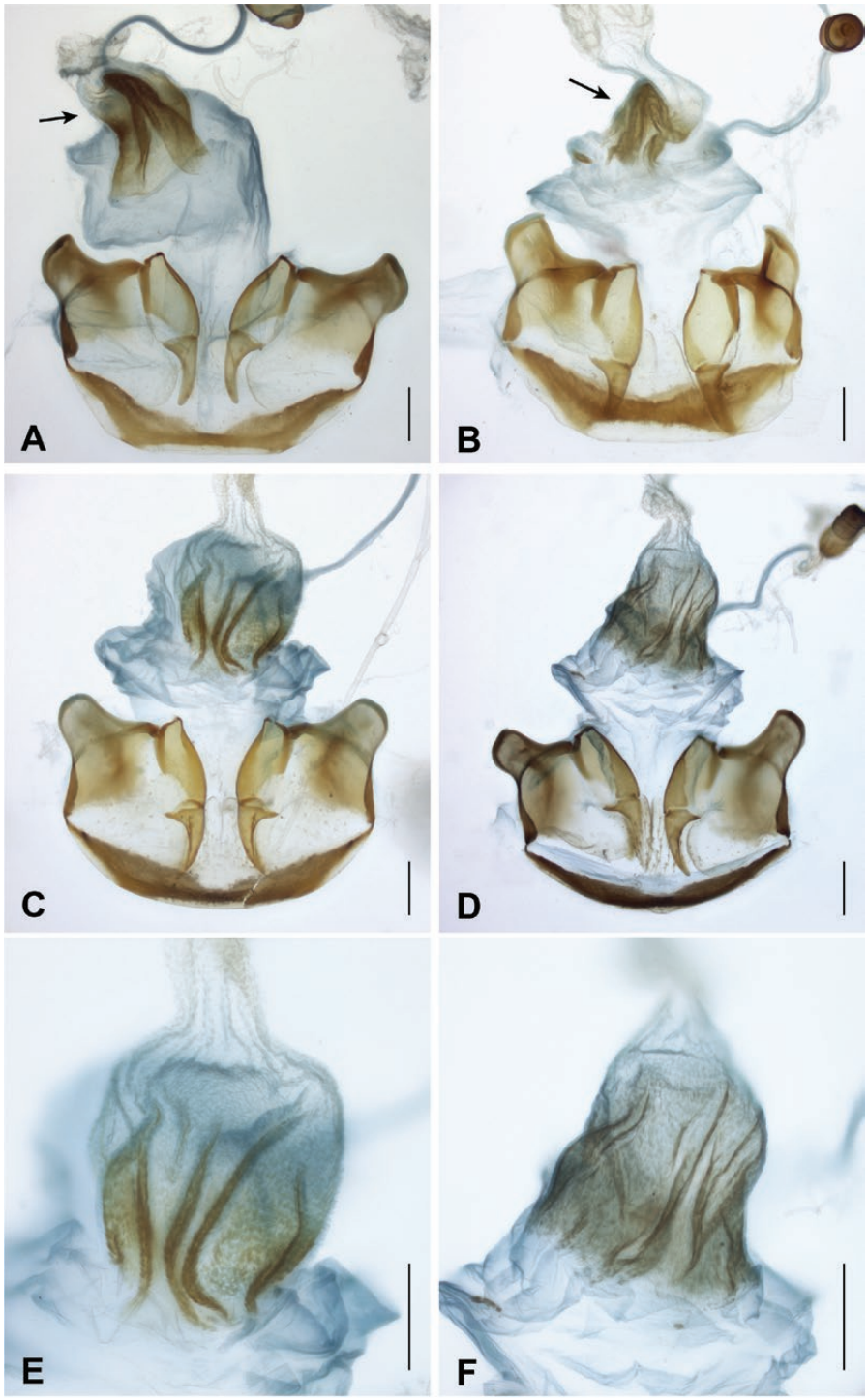

Figure 9. Female reproductive tract of the *Lionepha erasa* species group, dorsal view. Arrows in A and B indicate the dorsal microtrichial patch of the bursa. A, *L. probata*; B, *L. erasa*; C, *L. kavanaughi*; D, *L. lindrothi*; E, *L. kavanaughi*, closeup; F, *L. lindrothi*, closeup. Scale bars 100 μm.

CYTOGENETIC RESULTS

Eight of the species examined have 12 pairs of autosomes, and an X0/XX sex-chromosome system, for a complement of 25 chromosomes in males (Table 5). Preparations of the ninth species examined, *Lionepha pseudoerasa* (Lindroth, 1963), were insufficient to

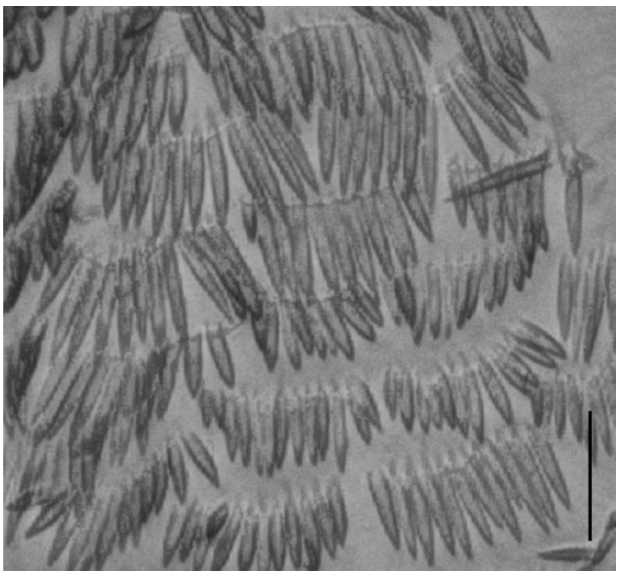

Figure 10. Photograph of microtrichia in the dorsal microtrichial patch of bursa of a female *L. casta*. Scale bar 10 µm.

determine the sex chromosomes, but the 25 chromosomes seen in the single male studied were consistent with results from other species. Re-examination of notes taken about the single male of *L. casta* examined by Maddison (1985) suggest that the tentative male count of 22+XY reported was in error.

DISCUSSION

Species delimitation

For a group containing species that are extremely difficult to tell apart morphologically, the DNA data tell a surprisingly clear story about species boundaries. In individual gene analyses, each species is strongly supported as monophyletic, with five to seven genes supporting monophyly, except for the similar pair of *L. erasa* and *L. australerasa* (Figs 5–7; Table 4). Monophyly of *L. erasa* is supported strongly by 28S and moderately strongly by *COI*, whereas *L. australerasa* is weakly supported as monophyletic only by 28S and *Topo*. However, the STACEY analysis (Fig. 8) clearly supports a structure consistent with each of the 11

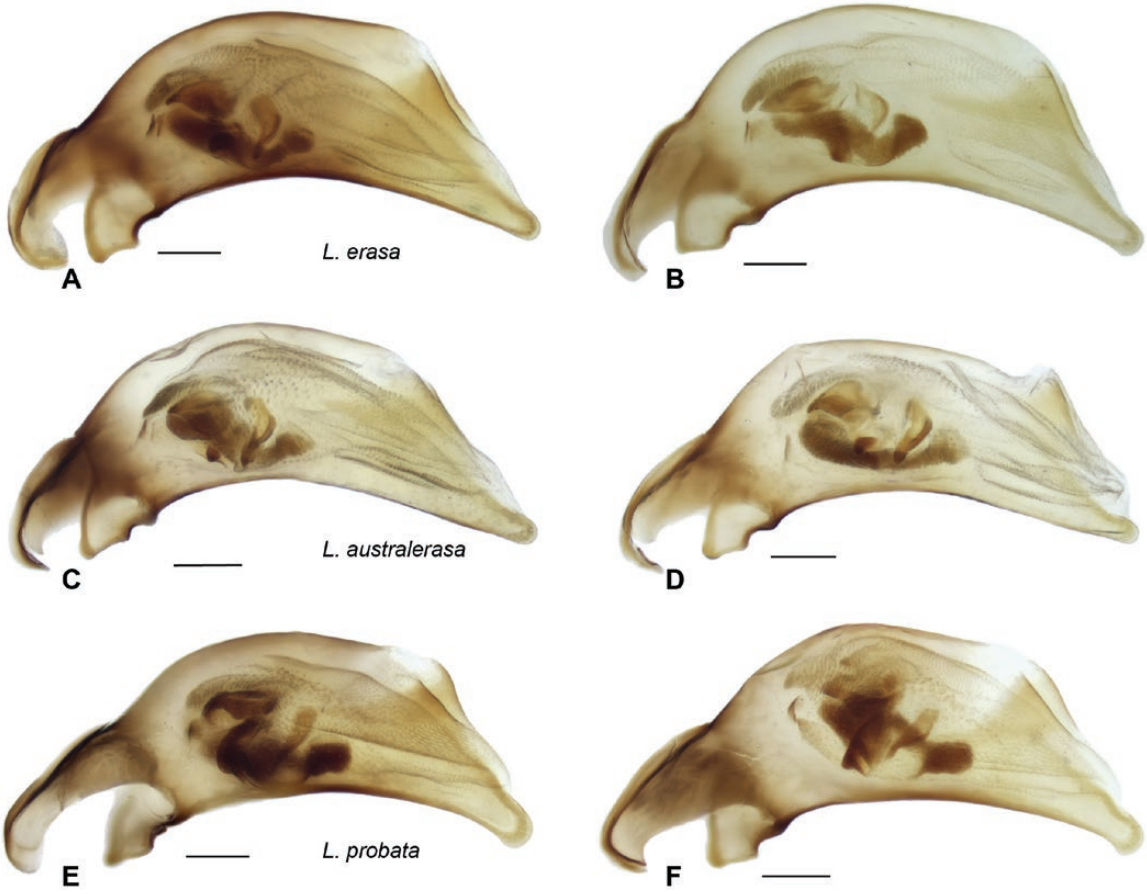

Figure 11. Aedeagus of three species of the *Lionepha erasa* species group, left lateral view. A, *L. erasa*; B, *L. erasa*; C, *L. australerasa*; D, *L. australerasa*, holotype; E, *L. probata*; F, *L. probata*. Scale bar 0.1 mm.

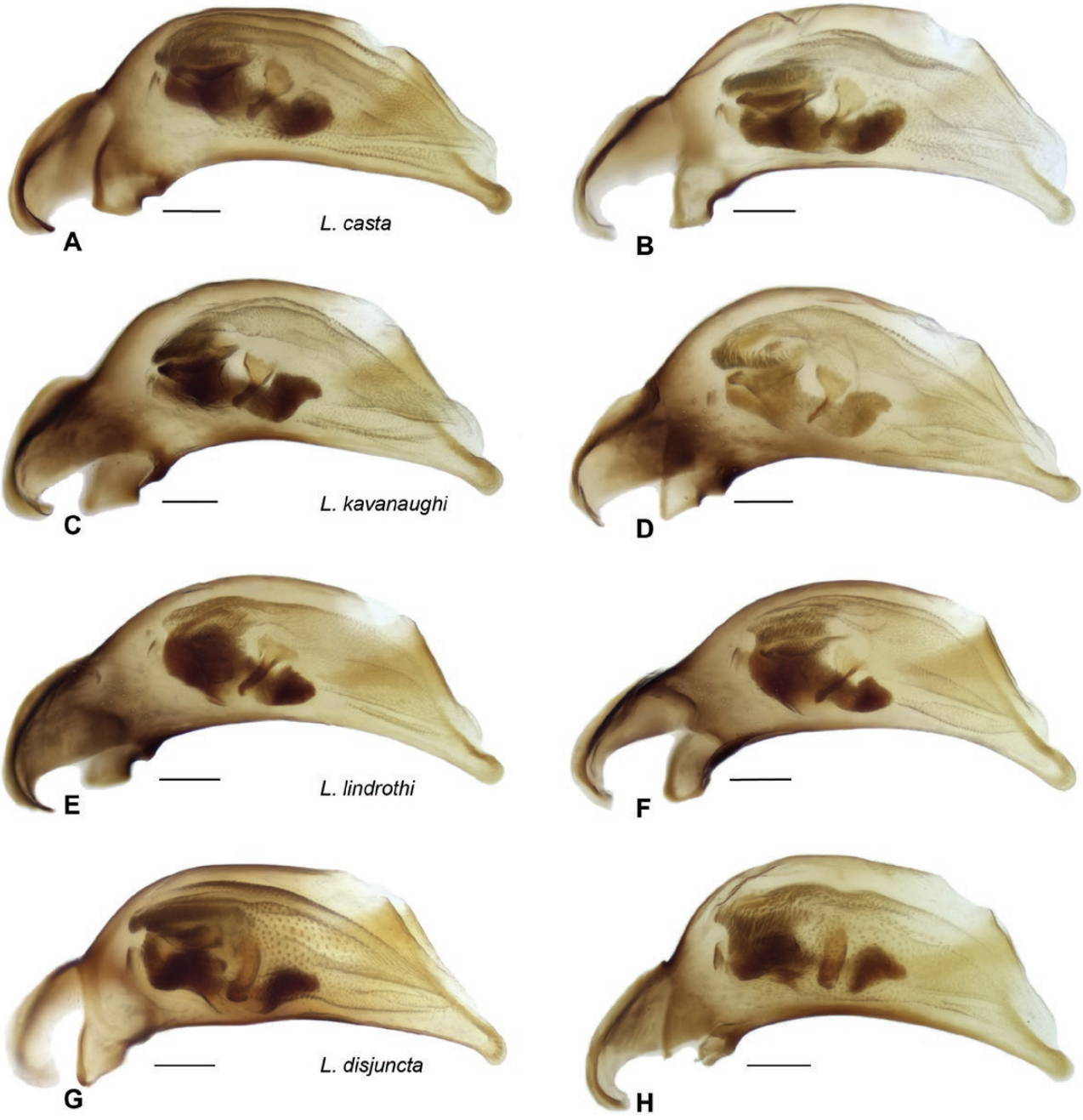

Figure 12. Aedeagus of four species of the *Lionepha erasa* species group, left lateral view. A, *L. casta*; B, *L. casta*; C, *L. kavanaughi*, holotype; D, *L. kavanaughi*; E, *L. lindrothi*, holotype; F, *L. lindrothi*; G, *L. disjuncta*; H, *L. disjuncta*. Scale bar 0.1 mm.

forms treated here as being separate species. Combined with corroboration provided by morphological results (see 'Taxonomic treatment', below), and extensive sympatry of the forms (Table 6), the data provide strong evidence for the distinctiveness of most proposed species of *Lionepha*. However, there are two species pairs of morphologically extremely similar, allopatric species that might be viewed as somewhat doubtfully separate: *L. casta* and *L. kavanaughi*, and *L. australerasa* and *L. erasa*.

For *L. casta* and *L. kavanaughi*, doubt about their distinctiveness could be based on their extremely similar genital structures. However, the phylogenetic analyses of the DNA data clearly indicate reciprocal monophyly (Figs 5–8; Table 4). In addition, there are DNA data not considered in the maximum likelihood

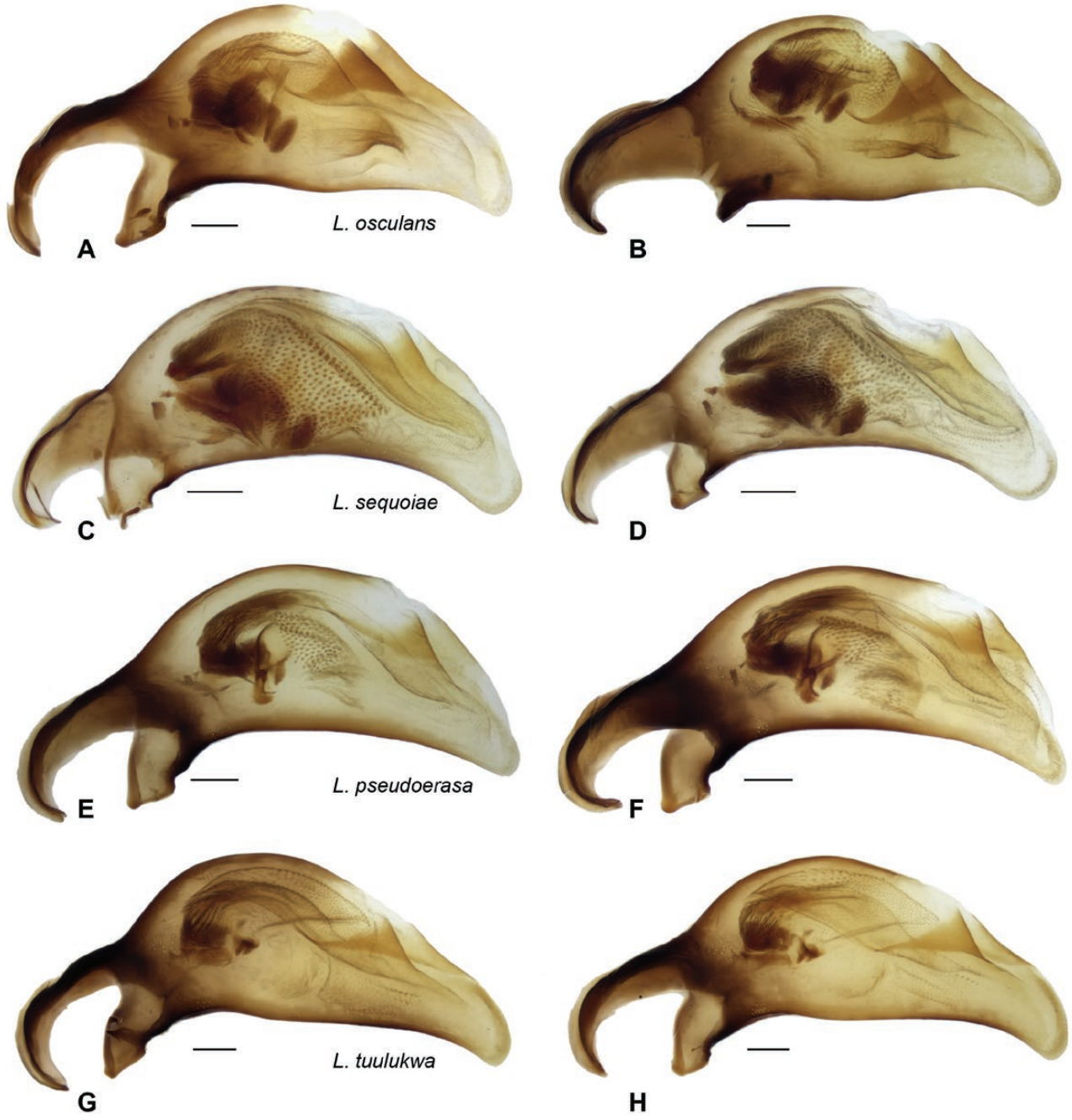

Figure 13. Aedeagus of the *Lionepha osculans* species group, left lateral view. A, *L. osculans*; B, *L. osculans*; C, *L. sequoiae*; D, *L. sequoiae*; E, *L. pseudoerasa*; F, *L. pseudoerasa*; G, *L. tuulukwa*; H, *L. tuulukwa*. Scale bar 0.1 mm.

and Bayesian analyses of relevance: all sequenced *L. casta* have four separate insertions in 28S (total of 14 bases) relative to all *L. kavanaughi*. These strong molecular results, combined with the slight but evident morphological differences in male genitalia and elytral striation, indicate that they should be treated as distinct species.

The distinctiveness of *L. australerasa* and *L. erasa* is less clear than that between *L. casta* and *L. kavanaughi*. In addition to the moderate evidence provided by phylogenetic analyses, there is a unique two-base insertion in all sequenced *L. erasa* that is not present in *L. australerasa* (Supporting information, Fig. S2). In addition, there are differences in

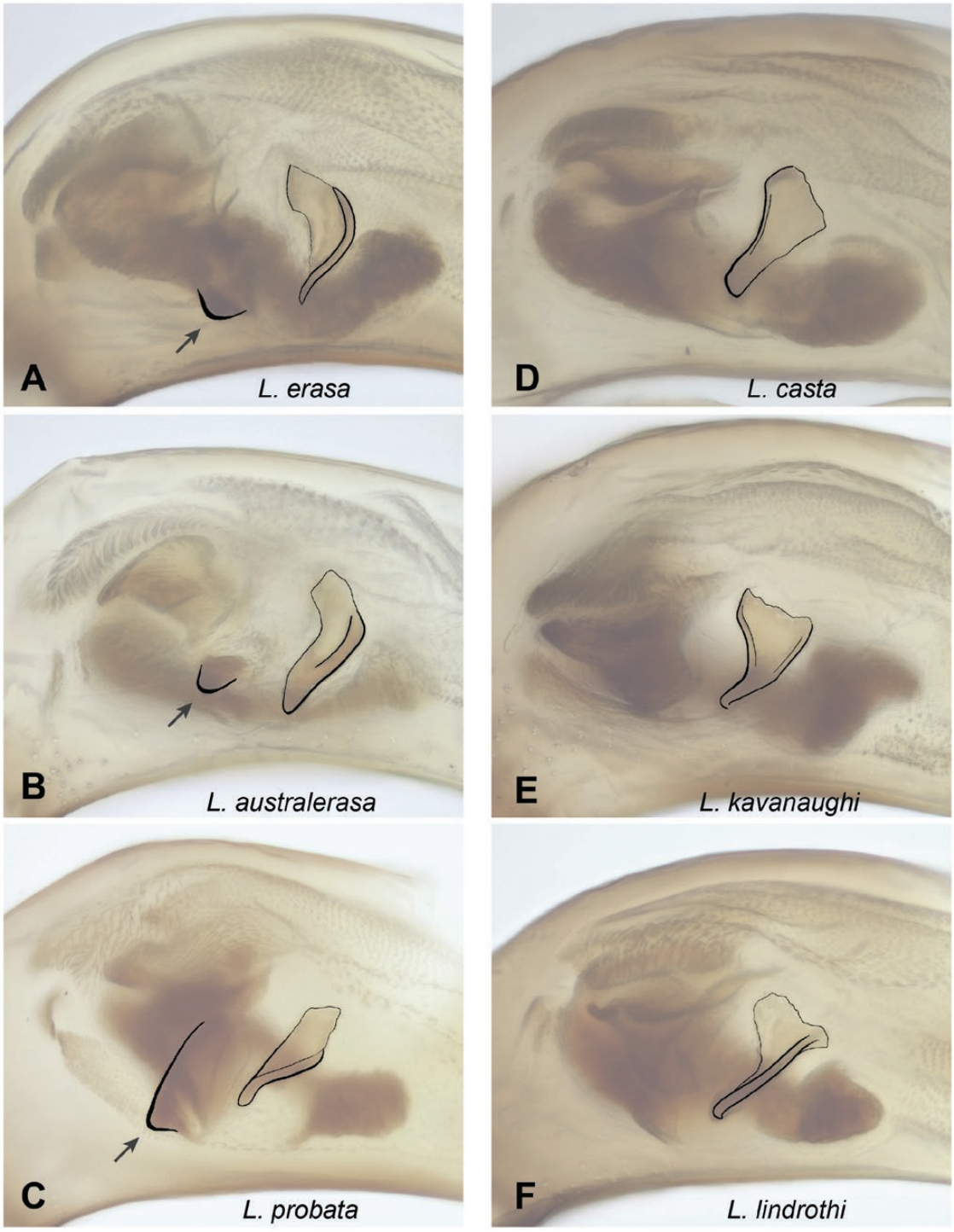

Figure 14. Aedeagus of six species of the *Lionepha erasa* species group, left lateral closeup view, with sclerite CH1 outlined, and the nub, when present, marked with an arrow. A, *L. erasa*; B, *L. australerasa*, holotype; C, *L. probata*; D, *L. casta*; E, *L. kavanaughi*, holotype; F, *L. lindrothi*.

microsculpture, colour and body form (including pronotal shape) between the two forms (see below). The evidence thus makes it reasonable to treat these forms as separate evolutionary lineages. A key test of this may come in populations in the Cascades of Oregon: if the forms can be found to be sympatric

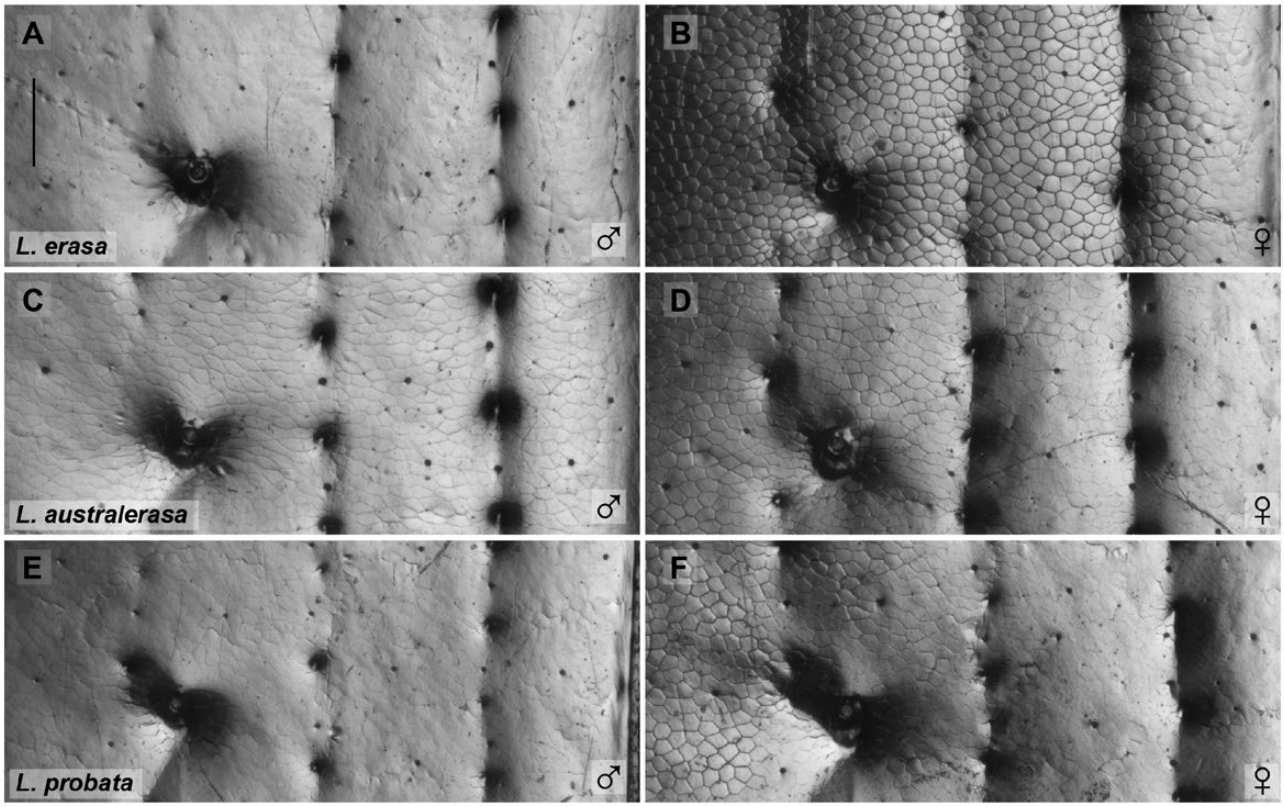

Figure 15. Microsculpture of the elytral disc of three species of the *Lionepha erasa* species group. Left elytron, region around anterior discal seta on third interval. A, *L. erasa* male; B, *L. erasa* female; C, *L. australerasa*, male; D, *L. australerasa*, female; E, *L. probata*, male; F, *L. probata*, female. Scale bar 50 µm.

(perhaps, for example, in the Pumice Desert of Crater National Park), their separateness might be confirmed or refuted.

TAXONOMIC TREATMENT

LIONEPHA

Lionepha Casey, 1918: 18.

Type species: Bembidium erasum LeConte, 1859, by original designation.

Diagnosis: Although DNA sequence data shows *Lionepha* to be clearly separate from the large genus *Bembidion* (Maddison, 2012; Maddison *et al.*, 2019), their morphological distinctiveness is not as evident. The arrangement of internal sac sclerites of male genitalia is perhaps the best defining character of the group, but we do not understand homologies of these structures to other bembidiines in sufficient detail to propose synapomorphies of either *Lionepha* or *Bembidion*. Female genitalic evolution is even more poorly understood in general, but *Lionepha* has a bursal characteristic that is likely derived. The dorsal surface of the bursa of female *Lionepha* has a region that is darker than the surrounding membrane, and is covered with a mat of microtrichia so dense that it is brown (Fig. 9). The microtrichia (Fig. 10) are arranged in short rows, point posteriorly and appear to be on the inside of the bursa. This brown region was called the 'dorsal sclerite' by Erwin & Kavanaugh (1981), but we call it the 'dorsal microtrichial patch'. We do not know of any other bembidiines with a similar structure, although the carabid genus *Dyscolus* (Platynini) has a dense microtrichial patch encircling the bursa (Moret, 1989). Within trechites, the dorsal microtrichial patch appears to be an autapomorphy of *Lionepha*. As such, it does not help to place the lineage. In external characters, *Lionepha* are no more distinctive than many subgenera of *Bembidion*.

Nonetheless, adult *Lionepha* can in general be recognized by the following suite of characters: they are moderate-sized bembidiines (3.3 to 6.0 mm) with unspotted, brown to black bodies. In body form, the smaller members are reminiscent of *Phyla* (currently considered a subgenus of *Bembidion*). Head with frontal furrows parallel, not deep and not prolonged onto clypeus; antennae short and thick. Mentum with full, more or less triangular epilobes and triangular or subtriangular

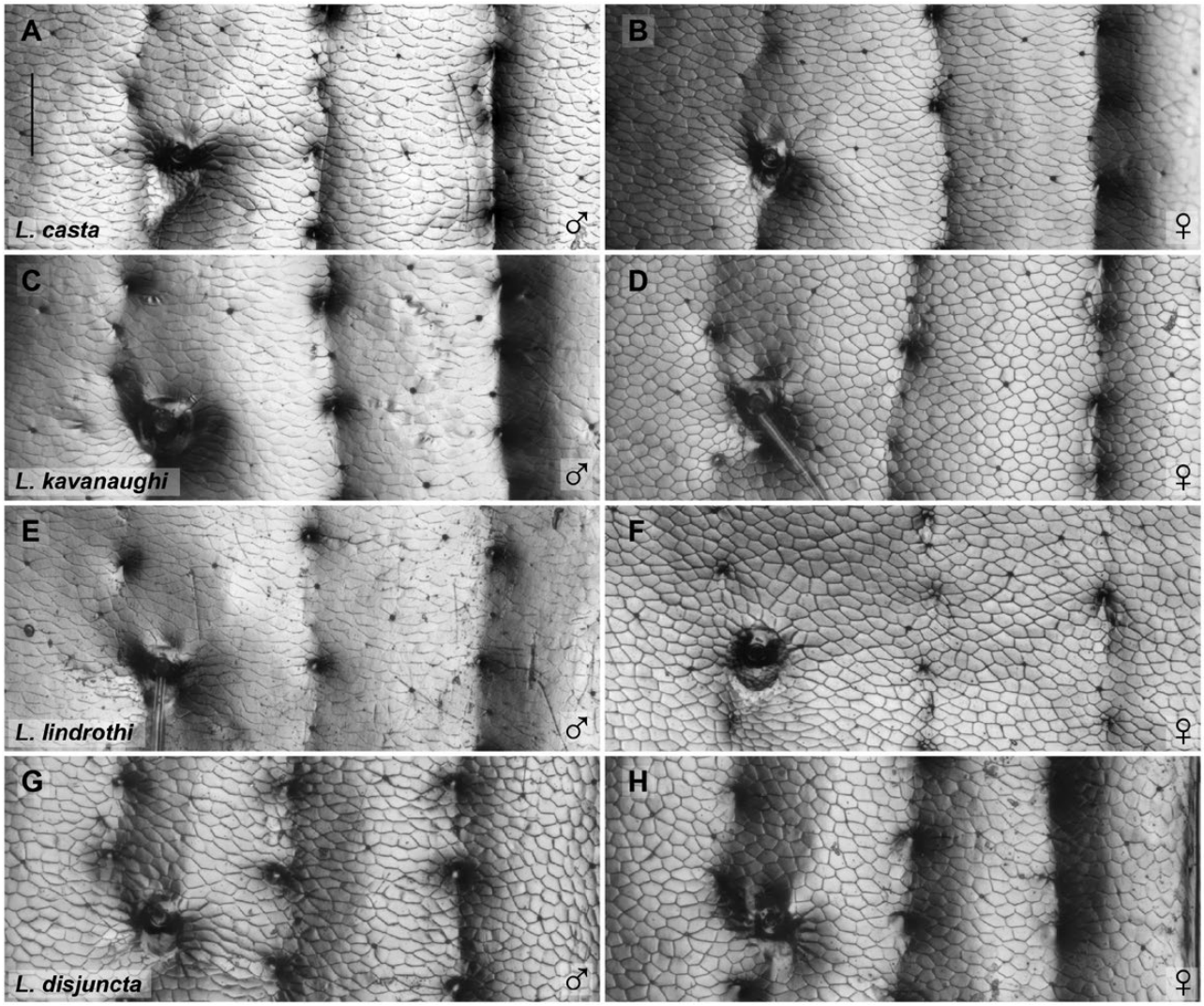

Figure 16. Microsculpture of the elytral disc of four species of the *Lionepha erasa* species group. Left elytron, region around anterior discal seta on third interval. A, *L. casta* male; B, *L. casta* female; C, *L. kavanaughi*, male; D, *L. kavanaughi*, female; E, *L. lindrothi*, male; F, *L. lindrothi*, female; G, *L. disjuncta*, male; H, *L. disjuncta*, female. Scale bar 50 µm.

mentum tooth. Pronotum with deep basolateral foveae, bounded externally by a strong, forward-converging carina. Lateral margin of elytra not prolonged inside shoulder; recurrent groove short, preapical seta (Ed7B) free; discal setae of elytra confluent with third stria; only the first elytral stria reaches elytral apex, striae 6–8 absent or nearly so. Metasternal process completely bordered. Hind wings in most species full, but dimorphic in *L. erasa* and *L. casta*. Female bursa with a dorsal microtrichial patch consisting of a dense, brown mat of microtrichia. Twelve pairs of autosomes, in contrast to the 11 pairs of the vast majority of *Bembidion*; males lack a Y chromosome (and thus *Lionepha* have an XO/XX sex chromosome system).

Larvae of *Lionepha* are not distinguishable from larvae of *Bembidion*. We have studied two first-instar larvae of *Lionepha casta* (obtained *ex ovo* from a culture of adults), as well as a single field-caught first-instar larva of *Lionepha erasa* (this is specimen DNA2586; see Figs 5, 6). *Lionepha* larvae have setae FR4 and FR5 closely approximate on the head, a synapomorphy of *Amerizus*, *Asaphidion* and *Bembidion* (Grebennikov & Maddison, 2005; Maddison, 2012), as well as all other features of *Bembidion* larvae documented by Grebennikov & Maddison (2005).

Lionepha is composed of two clades (Fig. 8): the *L. erasa* group, containing the smaller *Lionepha*, and

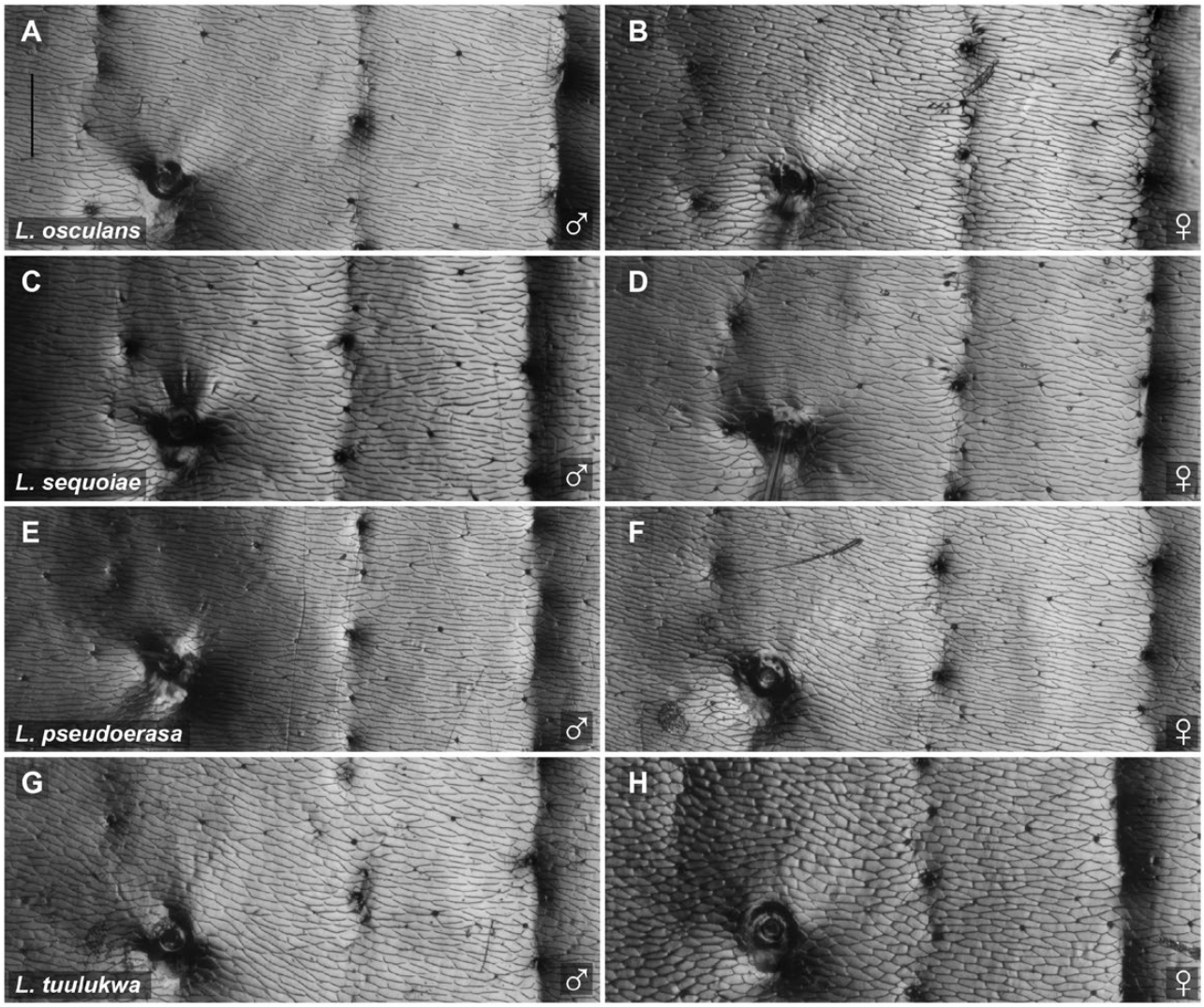

Figure 17. Microsculpture of the elytral disc of the *Lionepha osculans* species group. Left elytron, region around anterior discal seta on third interval. A, *L. osculans*, male; B, *L. osculans*, female; C, *L. sequoiae*, male; D, *L. sequoiae*, female; E, *L. pseudoerasa*, male; F, *L. pseudoerasa*, female; G, *L. tuulukwa*, male; H, *L. tuulukwa*, female. Scale bar 50 µm.

the *L. osculans* group, containing the four species with larger adults. These two groups have distinct male genitalia, with the *L. osculans* group having a broad apex, and the *L. erasa* group having a uniform pattern of internal sac sclerites (Figs 11–13).

The following species of *Lionepha* are now known (the novel taxa are described below):

L. erasa group

L. erasa (LeConte, 1859)
L. australerasa Maddison
L. probata (Casey, 1918)
L. casta (Casey, 1918)
L. kavanaughi Maddison
L. lindrothi Maddison & Sproul
L. disjuncta (Lindroth, 1963)

L. osculans group

L. osculans (Casey, 1918)
L. sequoiae (Lindroth, 1963)
L. pseudoerasa (Lindroth, 1963)
L. tuulukwa Maddison

Identification of species

Most *Lionepha* specimens are difficult to identify to species using external morphological structures. Although the majority of specimens can be identified with good equipment and by the trained eye, many are

only identifiable using male genitalia (Figs 11–14), elytral microsculpture (Figs 15–17), or DNA sequences. In most species, there are exceptional specimens that have prothoracic shape, elytral striae or other external characters that are outside the normal bounds of the species, and thus most species contain specimens that cannot be successfully identified using the external traits we have examined. In addition, the most important morphological characters, which are in male genitalia and elytral microsculpture, require special procedures or equipment to observe. The former requires clearing with KOH or enzymes and mounting in a medium with an appropriate refractive index (e.g. Euparal, clove oil or cedarwood oil). Within the *L. osculans* group, the male genitalia of each species are distinctive (Fig. 13). Within the *L. erasa* group, genitalia are much more similar, and natural variation in the exact position of internal sac sclerites makes comparisons more difficult, but the shape of sclerite CH1 (Fig. 14) is often sufficient for identification. A clear view of microsculpture requires a high-quality, powerful microscope and appropriate lighting (either a ring light or diffuse light if a dissecting scope is used, or coaxial illumination if a compound scope is used).

The large number of tenerals of *Lionepha* that are captured, more so than is typical for bembidiines, complicates identification. The reason for the high frequency of tenerals is unclear. Perhaps they emerge after eclosion earlier than other bembidiines, and are thus more accessible to collectors, or perhaps they are soft and pale for a longer period. Whatever the cause, tenerals of otherwise dark species will look like paler species (e.g.

KEY TO SPECIES OF *LIONEPHA*

1. Specimens smaller, body length 3.29–4.42 mm (few specimens longer than 4.2 mm). Microsculpture on elytra absent or consisting of isodiametric or somewhat transverse meshes (Figs 15, 16), but never transverse meshes or close-set transverse lines causing iridescence. Male genitalia with narrower apex, and with the characteristic pattern of internal sclerites shown in Figs 11, 12 ..... *L. erasa* group (2)
– Specimens larger, body length 4.26–5.90 mm (few specimens less than 4.4 mm). Microsculpture on elytra consisting of close-set transverse lines causing iridescence (e.g. Fig. 17A) or transverse meshes (e.g. Fig. 17C, D), or somewhat transverse (Fig. 17G, H); never isodiametric or absent. Male genitalia with broader apex (Fig. 13) ..... *L. osculans* group (9)
2. Body relatively flat. Prothorax small relative to elytra (Fig. 2D). Each elytron with at least five well-impressed striae, and with outer striae nearly effaced, but evident. Microsculpture of elytra deeply engraved, even in males (Fig. 16G), with slightly transverse or nearly isodiametric meshes ..... *L. disjuncta*
– Body relatively convex. Prothorax larger relative to elytra (Figs 1, 2A–C). Striae less well-developed in general. Microsculpture less deeply engraved on elytral disc of males (Figs 15A, C, E, 16A, C, E), isodiametric or transverse 3
3. Males shiny, with microsculpture on elytral disc weak or absent, isodiametric or slightly transverse (in *L. australerasa*, Fig. 15C; treated under both couplets). Microsculpture on elytra of females isodiametric, or only slightly transverse (at most as in Fig. 15B, D). Aedeagus with dark, distinct nub (arrows in Fig. 14). Female bursa with triangular or lobate dorsal microtrichial patch, much wider posteriorly than anteriorly (Fig. 9A, B) 4
– Microsculpture on elytral disc of males easily visible under high magnification with appropriate lighting, with clearly transverse sculpticells (Fig. 16A, C, E, G); microsculpture of females slightly transverse (Fig. 16B, D, F, H). Aedeagus without a nub. Female bursa with rectangular dorsal sclerotized region, not wider posteriorly than anteriorly (Fig. 9C, D) 6
4. Prothorax broader, with more rounded sides (Fig. 1C). Legs dark, piceous, nearly as dark as body. Male genitalia with ventral surface more curved, near apex more abruptly curved downward (and thus the apex expands towards the tip; Fig. 11E, F). Internal sac of aedeagus with dark nub large (Fig. 14C); sclerite CH1 as in Fig. 14C. Females with large dorsal microtrichial patch on bursa (Fig. 9A) ..... *L. probata*
– Prothorax narrower relative to elytra, with less rounded sides (Fig. 1A, B). Legs lighter than body, or as dark. Male genitalia with ventral surface straighter, with apex not expanded toward tip (Fig. 11A–D). Internal sac of aedeagus with smaller nub (Fig. 14A, B); sclerite CH1 as in Figures 14A and B. Females with smaller dorsal microtrichial patch on bursa (Fig. 9B) 5
5. Prothorax wider (PW/EL 0.467–0.478, >0.47 in most specimens), sides more rounded (Fig. 1A); hind angles in most specimens, therefore, more obtuse. Tibiae distinctly paler than body. Microsculpture of elytral disk isodiametric, or nearly so (rarely slightly transverse; Fig. 15E, F). Sclerite CH1 as in Figure 14A. From Oregon (Coast Range, central Cascades) north to Alaska ..... *L. erasa*

– Prothorax small, narrow (PW/EL 0.438–0.466, <0.46 in most specimens), more parallel-sided, sides straighter, especially posteriorly (Fig. 1B); in most specimens hind angles thus less obtuse. Tibiae dark, same colour as body. Microsculpture slightly stronger, evident on elytral disk, although faint in males; always slightly transverse (Fig. 15C, D). Sclerite CH1 as in Figure 14B. Sierra Nevada of California north to Crater Lake, Oregon ..... *L. australerasa*

6. Legs paler, with tibiae clearly paler than body. Dorsal microtrichial patch of female bursa rectangular, not narrowing anteriorly, with multiple, deep, parallel, longitudinal folds (Fig. 9C, E) 7

– Legs darker, as dark as, or nearly as dark as, the body; males shinier, with less-impressed sculpticells. Dorsal microtrichial patch of female bursa rectangular and not narrowing anteriorly, without deep, parallel folds, or triangular and lobate, much wider posteriorly than anteriorly (Fig. 9B, D, F) 8

7. Fourth stria nearly effaced, much less impressed than the first. Sclerite CH1 as in Figure 14D. Triangular scales on left-most membrane of internal sac (Fig. 18). In the Cascades and westward, as well as the northern and coastal areas of California ..... *L. casta*

– Fourth stria only slightly less impressed than the first. Sclerite CH1 as in Figure 14E. Left-most membrane of internal sac without scales. In the Blue Mountains and Wallowas of Oregon and Washington east to Montana and Wyoming ..... *L. kavanaughi*

8. Microsculpture of the elytra more deeply engraved, complete sculpticells evident in the male, although slightly effaced; females duller. Sclerite CH1 as in Figure 14F. Dorsal microtrichial patch of female bursa rectangular and not narrowing anteriorly (Fig. 9D) ..... *L. lindrothi*

– Microsculpture slightly weaker, often without complete sculpticells in males (Fig. 15C, D). Sclerite CH1 as in Figure 14B. Dorsal microtrichial patch of female bursa triangular and lobate, much wider posteriorly than anteriorly (Fig. 9B) ..... *L. australerasa*

9. Elytral microsculpture consisting of less transversely stretched meshes, in males with most sculpticells being about three times as wide as tall (Fig. 17G), in females brick-like and deeply engraved (Fig. 17H); thus, the elytra in females are quite dull. Prothorax narrow, only slightly wider than head. Aedeagus with reduced internal sac sclerites (Fig. 13G, H). Oregon Coast Range and Trinity Alps of California ..... *L. tuulukwa*

– Elytral microsculpture more transverse, with less of a tendency to form distinct sculpticells (Fig. 17A–F) 10

10. Elytra not iridescent. Males with small basal protarsomeres, only slightly wider than second protarsomere (Fig. 19B) ..... *L. sequoiae*

– Elytra slightly to notably iridescent because of transverse microsculpture. Males with large basal protarsomeres, much wider than second protarsomere (Fig. 19A, C) 11

11. Elytra with reduced elytral striation, with third stria not visible beyond posterior dorsal puncture. Elytra only slightly iridescent. Prothoracic sides less rounded (Fig. 3C), in most specimens with the posterior portion being almost parallel sided. Aedeagus with ventral surface gently curved (Fig. 13E, F) ..... *L. pseudoerasa*

– Elytra with at least third stria visible beyond posterior dorsal puncture, and second stria almost reaching apex. Elytra distinctly iridescent. Prothoracic sides more rounded (Fig. 3A). Aedeagus deep, with ventral surface sinuate (Fig. 13A, B) ..... *L. osculans*

the tibiae will be paler than a fully developed individual) and their genitalia (male or female) will be more difficult to study. The appendage colour described below pertains to fully developed individuals, not tenerals.

Species accounts

Lionepha erasa (LeConte, 1859)

(Figs 1A, 15A, B, 11A, B, 14A, 9B, 20)

Bembidium erasum LeConte, 1859: 83. Lectotype ♀, designated by Erwin & Kavanaugh (1981: 59), in MCZ (type # 5490), examined, including genitalia and DNA. Type locality: 'Oregon' (original citation), restricted to Fort Klamath, Klamath County, Oregon, USA, by Erwin & Kavanaugh (1981: 59). Although Fort Klamath is a reasonable type locality for Erwin & Kavanaugh's concept of *Lionepha erasa* (i.e. the species here called *Lionepha probata)*, the species to which the types of *Bembidium erasum* belong is not known from the southern half of Oregon. In order to move the type locality within the range of the current species, we here change the type locality to Marys Peak, Oregon (44.5104°N 123.5593°W); on this mountain, *Lionepha erasa* is the only known

species of *Lionepha* with isodiametric microsculpture. GenBank accession numbers for DNA sequences of the lectotype are MN401767, MN401952, MN402005, MN402129 and MN402322; accession number of sequence reads in NCBI's Sequence Read Archive is SRR5230423.

Bembidion lindrothellus Erwin & Kavanaugh, 1981: 61. Holotype ♂ in MCZ (type # 32549), examined, including genitalia and DNA. Type locality: Haines Highway Mile 31.5, Little Boulder Creek, Alaska, USA. New synonymy. GenBank accession numbers for DNA sequences of the lectotype are MN401768, MN401784, MN401905, MN401953, MN402006, MN402130, MN402255 and MN402323; accession numbers of sequence reads in NCBI's Sequence Read Archive are SRR5230400 and SRR5230401.

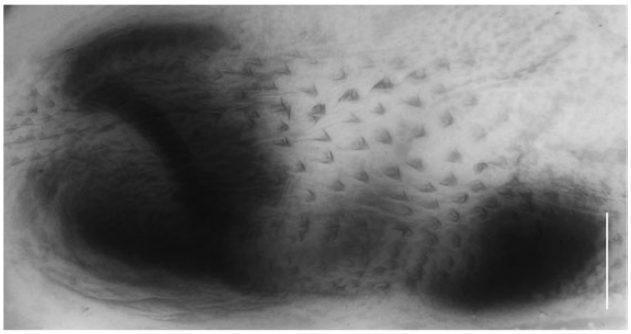

Figure 18. Leftmost membrane of internal sac of *Lionepha casta* to the left of sclerite CH1. Scale bar 50μm.

Bembidion lummi Erwin & Kavanaugh, 1981: 62. Holotype ♀, in CAS (type # 13652), examined. Type locality: Friday Harbour, San Juan Island, San Juan County, Washington, USA. Synonymy with *L. chintimini* established by Kanda *et al.* (2015).

Bembidion chintimini Erwin & Kavanaugh, 1981: 63. Holotype ♀, in CNC (type # 16452), examined. Type locality: Marys Peak, 1220 m, Benton County, Oregon, USA. New synonymy.

Nomenclatural notes: Among the non-type specimens we examined, all small *Lionepha* with isodiametric or effaced microsculpture from Oregon, Washington, Idaho, British Columbia (BC) and Alaska (the area containing the type localities of the four names listed above) belong to two species: a common, widespread species known from southern BC south to California, east to Montana and Colorado, but not known from Alaska or the Oregon Coast Range; and a rarer species known from the Oregon Coast Range, the Cascades of central and northern Oregon, north through Washington, BC, to near Anchorage, Alaska.

The realization that the four primary types of the above-listed names belong to only one of these species (the latter, rarer species) took several years, and was delayed by the fact that three of the primary types are females, one of which is teneral (the lectotype of *Bembidium erasum*),

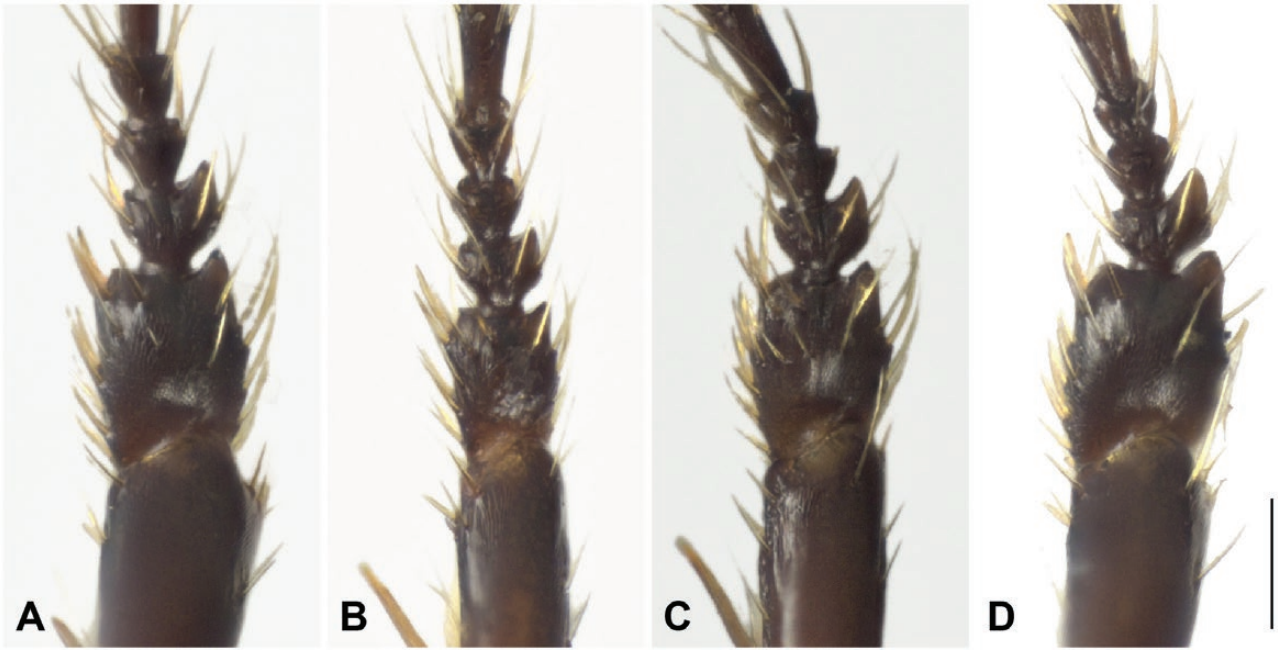

Figure 19. Dorsal view of male protarsi of the *Lionepha osculans* species group. A, *L. osculans*; B, *L. sequoiae*; C, *L. pseudoerasa*; D, *L. tuulukwa*.

Table 5. Chromosome numbers and sex chromosomes of *Lionepha* males. The Sample column indicates the number of males examined

	2n male	Sample	Locality	Reference
L. erasa	24+X	2♂	Oregon: Marys Peak	this paper
L. probata	24+X	1♂	Oregon: Lostine River Valley	this paper
L. casta	24+X	2♂	Oregon: Hospital Ck, Marys Peak	this paper
L. kavanaughi	24+X	2♂	Oregon: Lostine River Valley	this paper
L. disjuncta	24+X	1♂	Oregon: Lostine River Valley	this paper
L. sequoiae	24+X	1♂	Oregon: School Creek	this paper
L. osculans	24+X	2♂	California: Strawberry Creek	this paper
L. pseudoerasa	25	1♂	California: Strawberry Creek	this paper
L. tuulukwa	24+X	3♂	Oregon: Marys Peak	Pflug *et al.* in review

Table 6. Geographic proximity of species. Great-circle distances in kilometres between nearest known localities are shown, except if the specimens are known from the same geographic area, in which case the following symbols are used: S: sympatric, with overlapping ranges; M: microsympatric, collected by us within a few meters at a single locality

	L. erasa	*austra.*	*probata*	*casta*	*kavan.*	*lindrothi*	*disjuncta*	*osculans*	*sequoiae*	*pseudo.*
L. australeresa	167									
L. probata	**M**	**M**								
L. casta	**M**	**S**	**M**							
L. kavanaughi	193	461	**M**	220						
L. lindrothi	275	**S**	**M**	100	629					
L. disjuncta	**M**	**M**	**M**	**M**	**M**	**S**				
L. osculans	**S**	**M**	**M**	**M**	**M**	**S**	**M**			
L. sequoiae	**S**	**M**	**M**	**M**	335	**S**	**M**	**M**		
L. pseudoerasa	373	**S**	**M**	**M**	641	**M**	**M**	**M**	**M**	
L. tuulukwa	**S**	138	**S**	**M**	451	137	**M**	**M**	100	**M**

and the fourth type (holotype of *Bembidion lindrothellus*) is a teneral male with unpigmented, flattened genitalia.

Investigation of the rarer species, the one here called *Lionepha erasa*, began in 2010. Dissection of the first recognized males from Marys Peak, Oregon (type locality of *Bembidion chintimini*) revealed an aedeagus indistinguishable from those from San Juan Island, Washington (type locality of *Bembidion lummi*). The female holotype of *B. chintimini* is wingless and has slightly rounded shoulders. However, the Marys Peak population is wing-dimorphic, and winged individuals are in body form no different from the type series of *Bembidion lummi*. The elytral microsculpture of the holotype of *B. chintimini* is perfectly isodiametric (against Erwin & Kavanaugh, 1981), thus matching that of *B. lummi*. Other characters mentioned by Erwin & Kavanaugh as distinguishing the two populations are not consistent with available specimens. The lack of evident morphological differences, combined with effectively identical DNA sequences in specimens from Oregon, British Columbia and Alaska suggested that the Marys Peak populations are the same species as populations further north, and for this reason, *Bembidion chintimini* and *B. lummi* were synonymized by Maddison in Kanda *et al.* (2015).

This left in question the specimens considered to be *Bembidion lindrothellus* by Erwin & Kavanaugh, which are at first glance similar to the Marys Peak and other populations of '*Bembidion chintimini*'. Specimens classified as *Bembidion lindrothellus* are reported to be paler, but all specimens mentioned in Erwin & Kavanaugh (1981) are teneral. The unsclerotized aedeagus of the holotype of *Bembidion lindrothellus* made comparison of internal sac sclerites difficult. However, the internal sac membrane that rests in the left-most position has a species-specific microsculpture in *Lionepha*, and the microsculpture scales of the holotype of *Bembidion lindrothellus* from Alaska match those of Marys Peak specimens. A non-teneral male was also collected by Lindroth at the type locality of *B. lindrothellus*, but was not included in the type series, perhaps as the specimen was housed in Lindroth's collection in Lund, Sweden. This specimen is presumably the one whose genitalia Lindroth figured as *Bembidion brumale* (1963: fig. 127f). We have examined that specimen, and it is indistinguishable from specimens of

'*Bembidion chintimini*' from Alaska, British Columbia, Washington and Oregon, including details of the internal sac. Most critically, DNA sequences of the holotype of *Bembidion lindrothellus* are identical in eight studied genes to those of other specimens from throughout the range (Figs 5–7). It is thus evident that the holotypes of *Bembidion chintimini*, *B. lindrothellus* and *B. lummi* belong to a single species.

However, there is an older name. The type series of *Bembidium erasum* consists of four females. These specimens have traditionally been considered to belong to the common, widespread species here called *Lionepha probata*. Females of these two isodiametrically microsculptured species are difficult to tell apart, especially those with less-extreme prothoracic proportions (neither wide nor narrow). Although there are distinctions in the lobe of the female bursa of fully sclerotized individuals, interpretation of tenerals is more tenuous. Specimens in the type series of *Bembidium erasum* are all teneral, with prothoraces of moderate width, and thus there is no clear morphological evidence to place them to species. The type series was provided by George Suckley (LeConte, 1859), presumably captured during his travels as naturalist for the governor of Washington Territory during 1853–57 (Cooper & Suckley, 1859). The type series is from 'Oregon', which at the time encompassed the current area of Oregon, the southern half of what is now Idaho and some parts of Wyoming and Montana (Barry, 1932). Suckley's travels in Oregon included areas within the range of both species (Cooper & Suckley, 1859), and thus geography provides no clues about species membership. However, DNA data from the lectotype (and two of the paralectotypes; Sproul & Maddison, 2017) makes it clear that these specimens belong to the current species (Figs 5–7; Supporting Information, Fig. S1). Thus, the valid name of this species is *Lionepha erasa*, with *Bembidion chintimini*, *B. lindrothellus* and *B. lummi* as junior synonyms.

Diagnosis: A small, convex species (Fig. 1A) with isodiametric elytral microsculpture (Fig. 15A, B; effaced in most males) and pale tibiae contrasting against the darker body. Prothorax moderately wide [PW/EL 0.467–0.478, average 0.473 (N = 6), >0.47 in most specimens]. Aedeagus with straight ventral surface, with apex not expanded (Fig. 11A, B). Internal sac of aedeagus with distinct but small nub; sclerite CH1 as in Figure 14A. Female bursa with relatively small dorsal microtrichial patch (Fig. 9B).

Most similar to *L. australerasa*, but also difficult to distinguish externally from *L. probata*. Compared to both *L. australerasa* and *L. probata*, *L. erasa* is paler, with the tibiae distinctly paler than the body; *L. erasa* also tends to have a more convex body than in those species. In contrast to *L. australerasa*, sculpticells of the elytra are more perfectly isodiametric, although in a few specimens they are slightly transverse; the lateral margins of the pronotum of *L. erasa* are more rounded, with the sides distinctly converging posteriorly, and the hind angle always quite obtuse; the striae of elytra are slightly weaker, with smaller, less distinct punctures, often with only the three inner striae being clearly evident. Compared to *L. probata*, *L. erasa* has a narrower prothorax.

Additional characteristics: Body length 3.47–4.41 mm, although rarely above 4.2 mm. Antenna piceous. Legs with femora rufopiceous, and tibiae pale rufous or dark testaceous, except in specimens from the Cascades, whose tibiae can be rufopiceous. Hind wings dimorphic, with some individuals fully winged (e.g. DNA2615) and others brachypterous with narrow and sloped shoulders (e.g. DNA2616).

Geographic variation: Some individuals in populations from inland British Columbia have slightly transverse microsculpture (e.g. 15 km E New Denver, Zincton Summit, BC; MZLU) and are larger than typical for *L. erasa*. Specimens from the Cascades are darker, with darker tibiae, than specimens from the Oregon Coast Range.

The only notable variation in DNA sequences observed is in 28S: the two Alaskan specimens (DNA4059 and the holotype of *Bembidion lindrothellus*), as well as the lectotype of *Bembidion erasum*, are missing four bases ('ATTA') present in other specimens; this missing section occurs just after base 613 in the sequences of the holotype of *Bembidion lindrothellus* in GenBank (accession MN402323).

Note: This is the species referred to as *Bembidion brumale* in Lindroth (1963), but the type of *Bembidion brumale* is a member of *Lionepha casta*.

Distribution: Known from the Oregon Coast Range from Prairie Peak northward, as well as in the Cascades from Lost Prairie Campground, Oregon, northwards to Mt. St. Helens, at low elevation on the San Juan Islands and Vancouver Island, eastward to the western slopes of the Rocky Mountains and north to east of Anchorage, Alaska (Fig. 20); not known from south of 44.2°N. Found from 15 to 1500 m elevation. Collected in all months of the year except January, with lower frequency in midsummer; most commonly collected in the fall and early spring. In the Oregon Coast Range, where these beetles are found in high-elevation grasslands (e.g. on Marys Peak and Mt. Hebo), they are only active above ground once the rains start in August or September, until the rains cease in late spring. In June and July they are not in evidence, even at night.

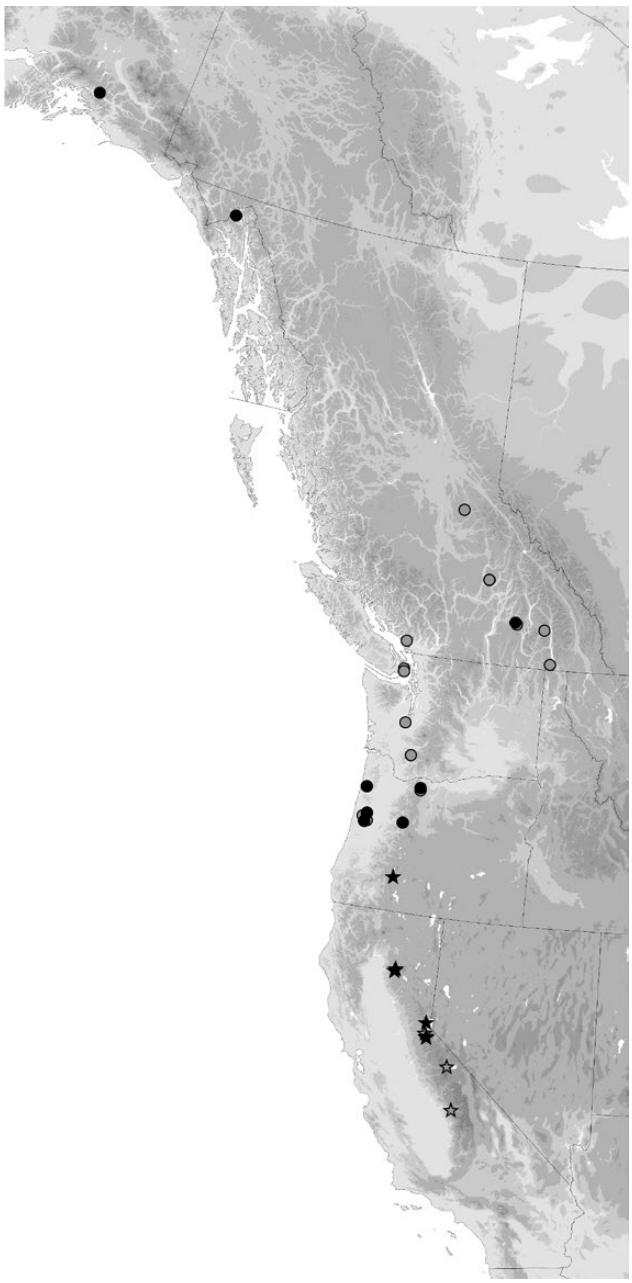

Figure 20. Geographic distribution of *L. erasa* (circles) and *L. australerasa* (stars). Specimens whose DNA was sequenced are shown in black.

Habitat: In the Oregon Coast Range, restricted to high-elevation grasslands (e.g. Fig. 4F), where they can be abundant under small rocks in the spring before the rains end, or early fall after the rains start. At Lost Prairie Campground in the Cascades of Oregon, found at the edge of snow melt in an open, grassy field; on Mt. St. Helens in Washington, found on the open pumice plain. Although typically found in open habitats away from water, a specimen has also been found along the shores of a stream (DNA4144 from Mount Hood, Oregon).

LIONEPHA AUSTRALERASA MADDISON, SP. NOV.

(FIGS 1B, 15C, D, 11C, D, 14B, 20)

http://zoobank.org/urn:lsid:zoobank.org:act:57CFECAE-CE5B-4D90-9839-59A71E0B3C07

Holotype ♂ (OSAC), herein designated, labelled: 'USA: California: Amador Co., Oyster Lake, Silver Lake Cpgd, 2205 m, 38.6711°N 120.1186°W, 31 May 2012. DRM 12.060. D.R. Maddison', 'David R. Maddison DNA3844 DNA Voucher' [pale green paper], 'HOLOTYPE *Lionepha australerasa* David R. Maddison' [partly handwritten, on red paper], 'Oregon State Arthropod Collection OSAC_0002000003 [matrix code]' [printed on both sides of white paper]. Genitalia mounted in Euparal in between coverslips pinned with specimen; extracted DNA stored separately. GenBank accession numbers for DNA sequences of the holotype are KY246650, KY246686, KY246720, KY246801, KY246842, MN401912, MN401959 and MN402263.

Paratypes (25): One paratype from the type locality (OSAC), as well as 24 specimens from the following localities: USA: California: Tulare Co., Sequoia Nat. Park, 6000', Huckleberry Meadow (2, MZLU); USA: California: Amador Co., Carson Spur, 2430 m, 38.7047°N 120.1055°W (3, OSAC); USA, California, Amador County, Highway 88 at Carson Spur, 2430 m, 38.70459°N 120.10554°W (1, CAS); USA: California: El Dorado Co., Martin Meadow, 2305 m, 38.6958°N 120.1223°W (1, OSAC); California: El Dorado Co., Strawberry Valley (1, CAS); California: El Dorado Co., 0.8 miles S of Sciots Camp at Strawberry Creek, 38.78329 120.14628 1745 m (1, CAS: CASENT1043929); USA: California: El Dorado Co., trail south of Lily Lake, 2012 m, 38.874°N 120.0804°W (1, OSAC); USA: California: Placer Co., creek in Homewood Canyon, 1930 m, 39.0783°N 120.1610°W (3, OSAC); USA: California: Tehama Co., Nanny Creek, Lassen NF, 1585m, 40.3696°N 121.5607°W (2, OSAC); USA: California: Tehama Co., tributary of Mill Creek at Hwy 89, 1996 m, 40.4210°N 121.5333°W (7, OSAC, USNM); USA: Oregon: Klamath Co., Munson Creek, Crater Lake NP, 1981 m, 42.8987°N 122.1343°W (2, OSAC).

Type locality: USA: California: Amador Co., Oyster Lake, Silver Lake Campground, 2205 m, 38.6711°N 120.1186°W

Etymology: The epithet is derived from the Latin *australis*, southern, and *erasum*, expunged, which is also the specific epithet of a similar species, *Lionepha*

erasa. This species is a southern, close relative of *Lionepha erasa*.

Diagnosis: A small, dark species with slightly transverse elytral microsculpture (Fig. 15C, D). The prothorax is small [PW/EL 0.438–0.466, average 0.45 (N = 9), <0.46 in most specimens] and relatively parallel-sided (Fig. 1B), with less obtuse hind angles. Aedeagus with straighter ventral margin, with apex not expanded (Fig. 11C, D). Internal sac with distinct nub; sclerite CH1 as in Figure 14B. Dorsal microtrichial patch of bursa relatively small, triangular, narrower anteriorly (as in Fig. 9B). The combination of transverse elytral microsculpture and having a nub in the aedeagus is unique within *Lionepha*.

Most similar to *L. erasa*, but also difficult to distinguish externally from *L. probata*. Compared to *L. erasa*, *L. australerasa* is darker, with tibiae piceous or black in most specimens. Sculpticells of elytra more transverse. Prothorax narrower with less rounded sides, with posterior regions more parallel-sided, and hind angle in most specimens less obtuse. Striae of elytra slightly stronger, with larger, more distinct punctures; striae 4 and 5 usually evident and occasionally outer ones as well. The two species share the same overall shape of the aedeagus (Fig. 11A–D), especially the straight, ventral margin and unexpanded apex. *L. erasa* and *L. australerasa* are not known to be sympatric, but their ranges may overlap in the Cascades of Oregon. Most easily distinguished externally from *L. probata* by the transverse microsculpture and the notably narrower prothorax, which is also more parallel-sided, but some *L. probata* approach *L. australerasa* in this regard. *L. australerasa* has a straighter ventral margin of the aedeagus, without an expanded apex. Females of *L. australerasa* have a smaller dorsal microtrichial patch on the bursa than those of *L. probata*. The internal sac sclerites, including the size of the nub and shape of sclerite CH1, are distinctive in each of the three species (Fig. 14A–C).

Although not closely related, *L. australerasa* is most similar in external form to the sympatric *L. lindrothi*, in that they both have dark legs, slightly or moderately transverse microsculpture on the elytra (although males have somewhat or notably effaced microsculpture, making the shape of sculpticells difficult to observe), and normal-sized or narrow prothoraces. They are difficult to distinguish externally, although *L. australerasa* has less rounded prothoracic margins and more effaced microsculpture. The lack of a nub in the internal sac of *L. lindrothi* and the shape of sclerite CH1 (Fig. 14) allows males to be distinguished; females can be distinguished by the triangular, anteriorly narrowed dorsal microtrichial patch in *L. australerasa* (as in Fig. 9B) compared to the rectangular dorsal microtrichial patch of *L. lindrothi* (Fig. 9D, F).

Additional characteristics: Body length 3.63–4.14 mm. Antenna piceous. Legs with femora piceous, with tibiae piceous or rufopiceous, rarely rufous. Hind wings full-sized. Chromosomes of male 24 + X (Table 5).

Note: Referred to as *Lionepha* 'Carson Spur' in Sproul & Maddison (2017). Specimens of this species were identified as '*Bembidion erasum*' by Erwin & Kavanaugh (1981).

Distribution: Known only from the Sierra Nevada of California and Crater Lake, Oregon (Fig. 20); not known from north of 42.9°N. A relatively high-elevation species, found from 1570 to 2925 m elevation. Specimens have been found in May, June and July.

Habitat: As with the previous species, this species is not closely tied to flowing water, being found on damp loam in forests or their clearings. At the type locality and at Carson Spur found near small patches of damp soil in an open forest, likely where snow had recently melted. At Crater Lake, Oregon, found at night in damp soil under bushes in the small flood plain of Munson Creek.

LIONEPHA PROBATA (CASEY, 1918)

(FIGS 1C, 15E, F, 11E, F, 14C, 9A, 21)

Bembidion probatum Casey, 1918: 22. Lectotype ♀, designated by Lindroth (1975), in USNM (type # 36817), examined. Type locality: Boulder County, Colorado.

Bembidion lubricum Casey, 1918: 21. Lectotype ♂, designated by Lindroth (1975), in USNM (type # 36820), examined (including genitalia). Type locality: Truckee, Nevada County, California. Synonymy established, under the name *Bembidion erasum*, by Lindroth (1963).

Bembidion lascivum Casey, 1918: 21. Lectotype ♂, designated by Lindroth (1975), in USNM (type # 36821), examined (including genitalia). Type locality: Lake Tahoe, Placer Country, California. Synonymy established, under the name *Bembidion erasum*, by Lindroth (1963).

Nomenclatural notes: This common and widespread species has long been known as *Lionepha* (or *Bembidion*) *erasa* (e.g. Lindroth, 1963; Erwin & Kavanaugh, 1981; Maddison, 2012). However, the lectotype of *Bembidium erasum* does not belong to this taxon (see nomenclatural notes under *Lionepha erasa*). We consider the three Casey names listed above to be synonyms. As first revisers of this group, we choose *Lionepha probata* to be the name for this species. We have sequenced one of the paralectotypes of *Bembidion probatum* (specimen USNMENT01114822) and have confirmed that it belongs to this species (Figs 5–7;

Supporting Information, Fig. S1).

Diagnosis: Specimens of this species are dark, with a broad prothorax with rounded sides (Fig. 1C) and with relatively effaced, isodiametric microsculpture on the elytra (Fig. 15E, F). As the elytral microsculpture can be nearly absent in many males, the shape of sculpticells can be difficult to observe. Ventral surface of the aedeagus curved, with apex slightly expanded (Fig. 11E, F). Internal sac of aedeagus with large, distinct nub; sclerite CH1 as in Figure 14C. The dorsal microtrichial patch of the female bursa is lobate or triangular, narrowing anteriorly, and is much larger than in *L. erasa* and *L. australerasa* (Fig. 9A). The broad prothorax is indicated by PW/EL values, ranging from 0.463 to 0.498, with an average of 0.476 (N = 8).

From the similar *L. erasa*, distinguished by the darker colour, especially of the tibiae, and by the broader prothorax (in most specimens), as well as characteristics of the male genitalia (Figs 11, 14) and bursal lobe of the female genitalia (Fig. 9). For differences with the similarly dark *L. australerasa*, see the diagnosis under that species. *Lionepha probata* specimens might also be confused with those of the sympatric *L. lindrothi*, but the latter has clearly transverse microsculpture and, in general, narrower prothoraces.

Lionepha probata is also similar in appearance to *Bembidion commotum* Casey, 1918, with which it is frequently microsympatric. In contrast to *L. probata*, *Bembidion commotum* has no elytral microsculpture on the disk, and nearly complete elytral striae. *Bembidion commotum* also has a metasternal process bordered only laterally.

Additional characteristics: Body length 3.29–4.16 mm. Antenna piceous. Legs piceous, in a few specimens with slightly paler, rufopiceous tibiae. Hind wings full-sized. Chromosomes of male 24 + X (Table 5).

Geographic variation: The 28S gene shows two forms that differ by two bases (positions 388 and 404 in the alignment deposited on Dryad). One form (having G at both sites) is eastern, from Colorado and Montana west to the White Mountains of California and the south-eastern corner of the Sierra Nevada, and in the north-west to the Warner, Steens, Wallowas and Blue Mountains. The other form occurs in the Cascades and much of the Sierra Nevada westward. The most distinctive populations are those in Utah, with the four Utah specimens having a one-base insertion in 28S; three of the specimens also differ at two sites from all other *L. probata*, with the fourth Utah specimen having double-peaks at both of those sites. The four Utah specimens form a distinct, well-supported clade in the *COI* tree (Fig. 5), as well as the STACEY analysis (Fig. 8).

Distribution: This widespread species is common from southern British Columbia south to the San Bernadino and San Jacinto Mountains near Los Angeles, east to Colorado (Fig. 21). It is found in the broadest elevation range of any *Lionepha* species, from 65 to 3400 m. Specimens have been collected from April through November, with the majority being found in July and August.

Habitat: Found in many habitats close to some form of water. This species is common around the edges of melting snowfields in the Sierra Nevada and Cascades, as well as on the shores of creeks in forested areas (especially among mosses on sand or silt on the upper bank), but it can also occur in other habitats, such as in leaf litter around pools in an open forest floor.

LIONEPHA CASTA (CASEY, 1918)

(FIGS 2A, 16A, B, 12A, B, 14D, 18, 22)

Bembidion castum Casey, 1918: 20. Lectotype ♂, designated by Lindroth (1975), in USNM (type # 36818), examined. Type locality: Santa Cruz Mountains, Santa Clara County, California.

Bembidion serenum Casey, 1918: 21. Lectotype ♀, designated by Lindroth (1975), in USNM (type # 36819), examined. Type locality: Arcata, Humboldt Country, California. Synonymy established by Lindroth (1963).

Bembidion brumale Casey, 1918: 22. Lectotype ♀, designated by Lindroth (1975), in USNM (type # 36823, specimen USNMENT01114819), examined (including genitalia and DNA). Synonymy established by Erwin & Kavanaugh (1981: 52), confirmed through DNA sequences. Type locality: Metlakatla, British Columbia. GenBank accession numbers for DNA sequences of the lectotype are MN402439, MN402246 and MN401772; accession number of sequence reads in NCBI's Sequence Read Archive is SRR5230408.

Bembidion vacivum Casey, 1918: 22. Lectotype ♀, designated by Lindroth (1975), in USNM (type # 36822), examined. Type locality: Skeena River at Terrace, British Columbia. Synonymy established, under the name *B. brumale* Casey, by Lindroth (1963: 262).

Bembidion nescium Casey, 1918: 30. Lectotype ♂, designated by Lindroth (1975), in USNM (type # 36845), examined. Type locality: Metlakatla, British Columbia. Synonymy established by Lindroth (1963).

Diagnosis: Specimens of this species have relatively pale tibiae, with evident, transverse microsculpture on the elytra; the elytral striae are less impressed, with the fourth stria much less impressed than the first. Sclerite CH1 as in Figure 14D. Females share with *L. kavanaughi* a dorsal microtrichial

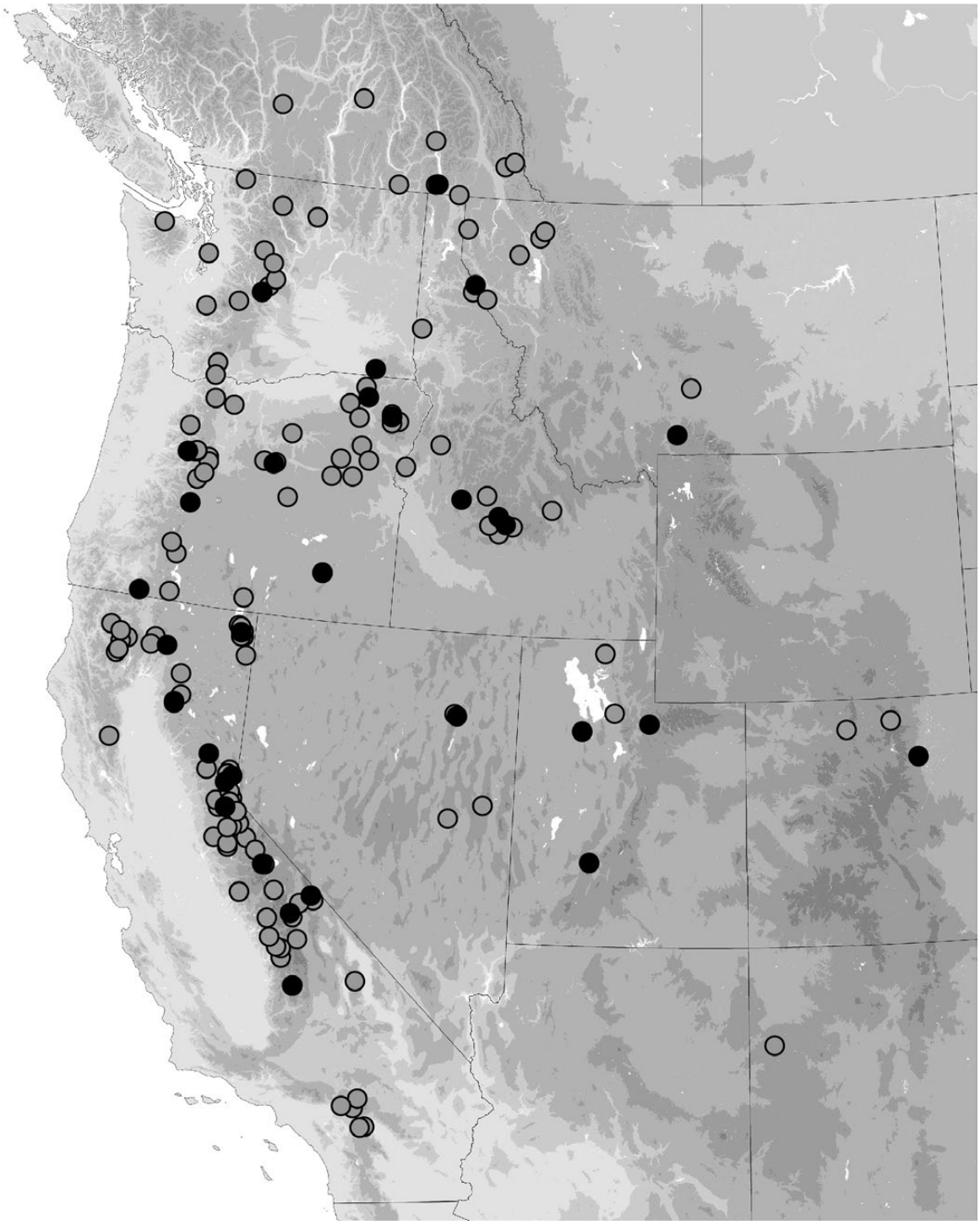

Figure 21. Geographic distribution of *L. probata*. Specimens whose DNA was sequenced are shown in black.

patch of the bursa that is rectangular (not narrowed anteriorly) and with deep, longitudinal, parallel folds (Fig. 9C).

This species is similar in appearance and genitalia to *Lionepha kavanaughi*, which with it shares pale tibiae, although the femora of *L. casta* are on average paler than in *L. kavanaughi*. However, *L. casta* has less impressed elytral striae. The most definitive characteristic of the male genitalia is the presence of distinct, triangular scales on the left-most membrane of the internal sac

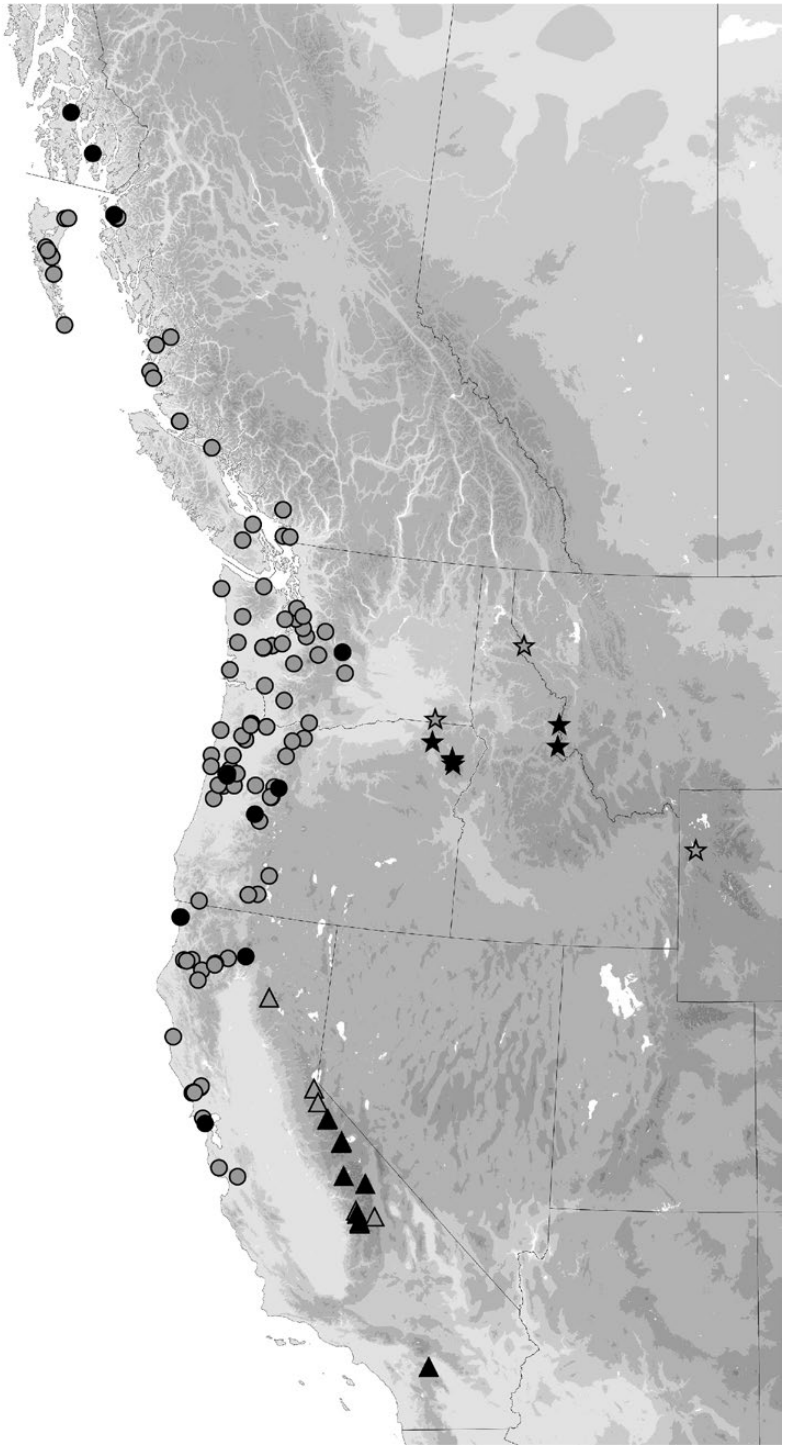

Figure 22. Geographic distribution of *L. casta* (circles), *L. kavanaughi* (stars) and *L. lindrothi* (triangles). Specimens whose DNA was sequenced are shown in black.

(Fig. 18); in contrast, there are no scales on membranes between sclerite CH1 and the outer, left wall of the aedeagus in *L. kavanaughi* or *L. lindrothi*. They are also geographically disjunct, with *L. casta* occurring only in the Cascades and westward (Fig. 22), and *L. kavanaughi* being found only in the north-east corner of Oregon and adjacent Washington east to Montana and Wyoming. From the sympatric *L. probata* it is most

easily distinguished by the narrower prothorax, paler legs and sculpticells that are clearly transverse.

Additional characteristics: Body length 3.45–4.22 mm. Antenna piceous. Femora rufous or rufopiceous; tibiae rufous or dark testaceous. Hind wings full-sized in most specimens, although a few brachypterous individuals are known (Erwin & Kavanaugh, 1981). Chromosomes of male 24 + X (Table 5).

Geographic variation: At the easternmost edge of the known range of this species (e.g. from Taneum Creek Campground in Wenatchee National Forest, Washington) specimens are unusually large and dark.

Distribution: One of the more widespread *Lionepha*, from coastal California north to southern Alaska (Fig. 22). It has not been found east of the Cascades. We agree with Erwin & Kavanaugh (1981) that Lindroth's (1963) record from Barkerville, BC, is doubtful. A lower elevation species, found between 0 and 1515 m, with the majority of specimens from below 200 m. It has been collected from March through November, with most specimens having been found in the summer.

Habitat: In numerous microhabitats often near water: on the sand or gravel shores of creeks in forests; in marshy areas along creeks in mountain forests. They can also be found during springtime in damp areas in high-elevation grasslands far from water.

LIONEPHA KAVANAUGHI MADDISON, **SP. NOV.**

(FIGS 2B, 16C, D, 12C, D, 14E, 9C, E, 22)

http://zoobank.org/urn:lsid:zoobank.org:act:B7402931-D60B-484A-8231-6DEB41460D52

Holotype ♂ (OSAC), herein designated, labelled: 'USA: Oregon: Wallowa Co., Lostine River Valley, 1483 m 45.3485°N 117.4152°W, 28 July 2016. DRM 16.069. D.R. & W.P. Maddison', 'David R. Maddison DNA5000 DNA Voucher' [pale green paper], 'HOLOTYPE Lionepha kavanaughi David R. Maddison' [partly handwritten, on red paper], 'Oregon State Arthropod Collection OSAC_0002000004 [matrix code]' [printed on both sides of white paper]. Genitalia mounted in Euparal in between coverslips pinned with specimen; extracted DNA stored separately. GenBank accession numbers for DNA sequences of the holotype are MN401771, MN401889, MN401946, MN401989, MN402108, MN402235, MN402308 and MN402428.

Paratypes (91): A total of 75 additional specimens from the type locality (deposited in OSAC, CAS, CNC, CMNH, USNM, MCZ, MNHN, MZLU, NHMUK, UASM, EMEC, JRLC), as well as 16 specimens from the following localities: USA: Oregon: Union Co., Little Phillips Creek at NF-3734, 1140 m, 45.6285°N 118.0163°W 16.072 (2, OSAC); USA: Oregon: Wallowa Co., Lostine River, Two Pan Trailhead, 1709m 45.249°N 117.3762°W (3, OSAC); USA: Oregon: Wallowa Co., Lostine River Valley, 1526 m, 45.3181°N 117.4015°W (1, OSAC); USA: Oregon: Wallowa Co., Lostine River, Two Pan Trailhead, 45.2490°N 117.3763°W, 1728 m (3, OSAC); USA: Oregon: Wallowa Co, Lostine River (1, OSAC); USA: Washington: Blue Mountains, Lewis Peak (1, OSAC); USA: Montana: Ravalli Co., Nez Perce Fork of Bitterroot River, 3.1 miles E of Nez Perce Pass on Nez Perce Pass Road, 45.73086°N 114.48828°W, 1785 m (1, CAS); USA: Montana: Ravalli Co., Lost Horse Creek, 17.1 miles W of Highway 93 on Lost Horse Road 46.14142°N 114.48584°W, 1752 m (1, CAS); USA: Montana: Ravalli Co., Lost Horse Creek, 12.3 miles W of Highway 93 on Lost Horse Creek, 46.13404°N 114.39418°W, 1513 m (1, CAS); USA: Montana: Sanders Co., Prospect Creek, 5.1 miles E Thompson Pass, 47.57539°N 115.64034°W (1, CAS: CASENT 1048646); USA: Wyoming: Grand Teton National Park (1, UASM).

Type locality: USA: Oregon: Wallowa Co., Lostine River Valley, 1483m, 45.3485°N 117.4152°W. The type locality is along a small tributary of the Lostine River, both above and below where it is crossed by Upper Lostine Road (Fig. 4D).

Etymology: It gives the first author great pleasure to name this species for David H. Kavanaugh, a superb carabidologist, good friend and collector of the first recognized specimens of this species.

Diagnosis: Specimens of this species have pale tibiae and evident, transverse microsculpture on the elytra (Fig. 16C, D); the elytral striae are relatively impressed (Fig. 2B), with the fourth stria similar in depth to the first. Aedeagus (Fig. 12C, D) without nub on internal sac (Fig. 12E); sclerite CH1 as in Figure 14E. Females share with *L. casta* a dorsal microtrichial patch of the bursa that is rectangular (not narrowed anteriorly) and with deep, longitudinal, parallel folds (Fig. 9C).

This species is similar in appearance and genitalia to *Lionepha casta*, which with it shares pale tibiae. However, *L. kavanaughi* has more impressed elytral striae, especially the fourth. They are most easily distinguished by the left-most membrane of the internal sac: *L. kavanaughi* lacks the obvious triangular scales present in *L. casta* (Fig. 18). *L. kavanaughi* occurs further east than any known localities for *L. casta* (Fig. 22).

Additional characteristics: This is the largest member of the *erasa* group, with a body length of 3.81–4.42mm. Antenna piceous. Femora piceous or rufopiceous; tibiae

paler, rufous or dark testaceous. Hind wings full-sized. Chromosomes of male 24 + X (Table 5).

Geographic variation: The two specimens sequenced from Montana are reconstructed as somewhat distinct in the STACEY tree (Fig. 8). These specimens differ by three bases in *COI* and three bases in *wg*, all representing synonymous differences in those protein-coding genes.

Note: Referred to as *Lionepha* 'Bitterroots' in Sproul & Maddison (2017). The specimen referred to in Erwin & Kavanaugh (1981: 54) as an 'Anomalous record' of *Lionepha casta* from Lewis Peak, Blue Mountains, Washington, is a female of this species.

Distribution: Known from the Bitterroot Mountains along the Montana–Idaho border and Grand Teton National Park in Wyoming, west to the Wallowas and Blue Mountains of north-eastern Oregon and south-eastern Washington. It likely occurs throughout the intervening regions of Idaho, but it has not yet been recorded there. There is a single female labelled 'Up. Truckee R 8-19-52 PSBartholomew' in CAS that externally appears most like *L. kavanaughi*, and it has a folded microtrichial patch matching that of *L. kavanaughi* or *L. casta*, but it is far enough outside the known distribution of either *L. kavanaughi* or *L. casta* that we consider the record doubtful, and have not included it on the map. Elevational range of the few known localities is between 1085 and 2060 m. Specimens were collected in June and July.

Habitat: In the Wallowa and Blue Mountains of Oregon, found on the gravel and sand shores of small creeks in forests (e.g. at the type locality, shown in Fig. 4D). *Lionepha probata* and *L. disjuncta* are also abundant at the type locality, along with *Bembidion kuprianovii* Mannerheim.

LIONEPHA LINDROTHI MADDISON & SPROUL, SP. NOV.

(FIGS 2C, 16E, F, 12E, F, 14F, 9D, F, 22)

http://zoobank.org/urn:lsid:zoobank.org:act:65FA8199-C515-4764-9E43-D0DA1D1DC973

Holotype ♂ (OSAC), herein designated, labelled: 'USA: California: Tulare Co., outlet of Emerald Lake, 2814m, 36.599°N 118.677°W, 21 June 2014. JSS 2014.065. J.S. Sproul & E.C. Sproul', 'David R. Maddison DNA4117 DNA Voucher' [pale green paper], 'SEQUOIA AND KINGS CANYON NATIONAL PARKS SEKI 23093' [green paper], 'HOLOTYPE Lionepha lindrothi Maddison & Sproul' [partly handwritten, on red paper], 'Oregon State Arthropod Collection OSAC_0002000005 [matrix code]' [printed on both sides of white paper]. Genitalia mounted in Euparal in between coverslips pinned with specimen; extracted DNA stored separately. GenBank accession numbers for DNA sequences of the holotype are MN401861, MN401929, MN401978, MN402081, MN402207, MN402287 and MN402400.

Paratypes (48): In addition to five specimens in OSAC from the type locality, the paratypes are from the following localities: USA: California, Riverside Co., Long Valley Creek along trail to Round Valley, 2585–2740 m, 33.81111°N 116.65062°W to 33.80346°N 116.65971°W (1, CAS); USA: California: Tulare Co., outlet of Emerald Lake, 2816 m, 36.5988°N 118.6772°W (4, OSAC); USA: California: Tulare Co., Mt. Silliman, Twin Lakes, 9800 ft (1, CAS); USA: California: Tulare Co., Sequoia Nat Park, Mineral King, 7700' (1, MZLU); USA: California: Tulare Co., snowfield below White Chief Lake, 2912 m, 36.417°N 118.5941°W (2, OSAC); USA: California: Sequoia National Park (2, CAS, MZLU); USA: California: Tulare Co., East Fork Kaweah River, 2377 m, 36.4502°N 118.5956°W (1, OSAC); USA: California: Tulare Co., East Fork Kaweah River, 2812 m, 36.4189°N 118.5927°W (6, OSAC); USA: California: Inyo Co., Mt. Whitney Trail below Lone Pine Lake, 2900 m (6, CAS, USNM); USA: California: Inyo Co., South Fork Bishop Creek, 2835 m, 37.1843°N 118.5585°W (1, OSAC), USA: California: Fresno Co., creek below Kaiser Pass, 2722m, 37.2865°N 119.1009°W (1, OSAC); USA: California: Mono Co., pond above Tioga Lake, 3016 m, 37.9125°N 119.2538°W (1, OSAC); USA: California: Mono Co., Ellery Lake, 2925 m, 37.9408°N 119.2432°W (1, OSAC); USA: California: Tuolumne Co., creek near Sonora Pass, 2831 m, 38.3337°N 119.6552°W (1, OSAC); USA: California: Tuolumne Co., Deadman Creek, 2700 m, 38.3188°N 119.6634°W (1, OSAC); USA: California: Tuolumne Co., Sonora Pass, 2815 m (1, CTVR); USA: California: Calaveras Co., Lampson's (?) Ranch 10 July 1907 (1, CAS); USA: California: El Dorado Co., Lily Lake, 2060 m, 38.8743°N 120.0819°W (9, CAS); USA: California: Alpine Co., Blue Lakes (1, CAS); USA: California: Shasta Co., Lassen Volcanic NP, Cascade Range, s. slope Mt. Lassen, Emerald Lake, 2450 m (1, CAS).

Type locality: USA: California: Tulare Co., outlet of Emerald Lake, 2814 m, 36.599°N 118.677°W.

Etymology: We are honoured to name this species after Carl H. Lindroth (1905–79), who transformed our understanding of bembidiines in North America through his great work of 1963.

Diagnosis: Specimens of this species are dark, with legs as dark as the body or nearly so, with a normal-sized prothorax (Fig. 2C), and with transverse microsculpture on the elytra (Fig. 16), although quite

weak (partly effaced) in males. Aedeagus (Fig. 12E, F) without nub in internal sac (Fig. 14F); sclerite CH1 as in Figure 14F. Females (Fig. 9D, F) have a dorsal microtrichial patch on the bursa that is rectangular (not narrowed anteriorly), without deep, longitudinal, parallel folds.

Lionepha lindrothi is similar to *L. casta* and *L. kavanaughi*, but females lack the deep, parallel folds of the dorsal microtrichial patch of the bursa of those species (compare Fig. 9D, F with Fig. 9C, E). In addition, *L. lindrothi* has darker legs and a distinctive sclerite CH1. As far as known, their ranges do not overlap, with *L. lindrothi* being the only one of the three in the Sierra Nevada and on Lassen Peak. It is possible that they co-occur on Mt. Shasta or Lassen Peak.

Although not closely related, this species is similar in appearance to the sympatric *Lionepha australerasa*, which with it shares dark legs and transverse microsculpture. However, *L. lindrothi* has a stronger microsculpture and a slightly larger prothorax; it also lacks the nub on the internal sac; for more details, see the diagnosis of *L. australerasa*.

Additional characteristics: Body length 3.48–4.20 mm. Antenna piceous. Legs piceous, although in some specimens the tibiae are rufous. Hind wings full-sized.

Geographic variation: The single specimen from the San Jacinto Mountains (DNA5072) has four unique bases in *COI* representing synonymous differences.

Note: Referred to as *Lionepha* 'Bishop Creek' in Sproul & Maddison (2017). Specimens of this species were identified as '*Bembidion erasum*' by Erwin & Kavanaugh (1981).

Distribution: Known only from Lassen Peak, the Sierra Nevada and the San Jacinto Mountains of California (Fig. 22). A higher-elevation species, found from 2000 to 3015 m, with the majority of specimens found above 2800 m. Collected in May through August.

Habitat: Found along creek and lake shores at high elevation (Fig. 4C).

LIONEPHA DISJUNCTA (LINDROTH, 1963)

(FIGS 2D, 16G, H, 12G, H, 23)

Bembidion disjunctum Lindroth, 1963: 264. Holotype ♂ in MCZ (type # 32533), examined. Type locality: Sonora Pass, Tuolumne County, California.

Diagnosis: A flat, parallel-sided species with more complete striae than other *Lionepha* (Fig. 2D), and with relatively strong, slightly transverse elytral microsculpture (Fig. 16G, H). Aedeagus (Fig. 12G, H) similar to other members of the *erasa* group, with a more or less ovoid CH1 sclerite.

In appearance *L. disjuncta* resembles a small member of *Bembidion* subgenus *Plataphus*, or a *Bembidion nebraskense* LeConte, 1863. From sympatric *Bembidion* (*Plataphus*) specimens it can be distinguished externally by the completely bordered metasternal process (bordered at the sides only in *Plataphus*). The elytral striae are less distinct in *L. disjuncta*. In *Plataphus*, multiple striae will be easily evident near the elytral apex. The aedeagus is different from *Plataphus*, without the evident flagellum present in members of that subgenus. From *Bembidion nebraskense* it can be immediately distinguished by the presence of elytral microsculpture: *B. nebraskense* lacks elytral microsculpture and is thus glossy.

Additional characteristics: Body length 3.62–4.32 mm. Antenna piceous. Femora rufopiceous or piceous, tibiae rufopiceous or rufous. Hind wings full-sized. Chromosomes of male 24 + X (Table 5).

Geographic variation: This species shows notable variation in 28S, with numerous insertion and deletion events evident (Supporting Information, Fig. S1). Of the ten specimens sequenced, seven have unique indels, and the remaining three share an eighth pattern of insertions and deletions. In addition, the specimen from the Wallowas that was sequenced has a unique amino acid, threonine, within *COI*, although other *L. disjuncta* have alanine at that position (base 356 in the sequence of specimen DNA3848, GenBank accession number MN402196).

Distribution: A widespread species, from the northern Sierra Nevada and Trinity Alps of northern California, north to southern British Columbia and east to Montana (Fig. 23). Found between 650 and 2930 m in elevation. Most records are from late summer and early fall, with specimens having been collected in June through September, as well as one record from April.

Habitat: This species is usually encountered as a specimen or two amongst more common *Bembidion* on gravel and cobble shores of cold, clear creeks and rivers. There are two localities where numerous specimens have been found: Summit Creek west of Creston, British Columbia, and in the Lostine River valley in the Wallowas of Oregon. At the latter locality,

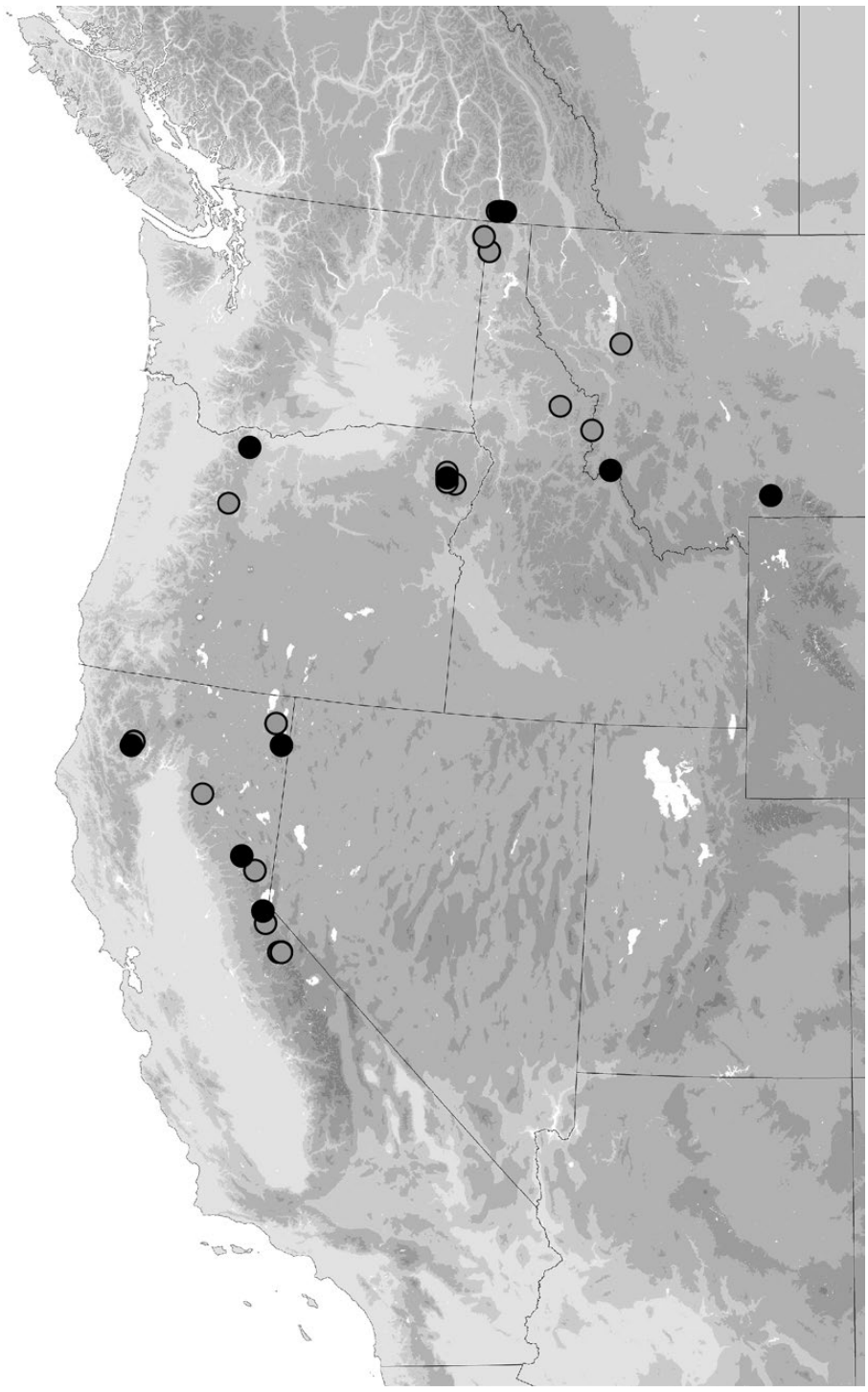

Figure 23. Geographic distribution of *L. disjuncta*. Specimens whose DNA was sequenced are shown in black.

36 specimens were found in the drying bed of a small creek in a forest (Fig. 4D), along with numerous *Lionepha kavanaughi* and *L. probata*, as well as *Bembidion kuprianovii*.

LIONEPHA OSCULANS (CASEY, 1918)

(FIGS 3A, 17A, B, 13A, B; 19A, 24A)

Bembidion osculans Casey, 1918: 20. Lectotype ♀, designated by Lindroth (1975: 116), in USNM (type

36816), examined. Type locality: Marin County, California, as restricted by Lindroth (1963).

Bembidion speculum Casey, 1918: 20. Lectotype ♀, designated by Lindroth (1975: 116), in USNM (type # 36815), examined. Synonymy established by Lindroth (1963). Type locality: Marin County, California.

Diagnosis: This is the largest species of *Lionepha*, with some females reaching nearly 6 mm in length. It is also the broadest, with a wide, rounded prothorax (Fig. 3A). The elytral microsculpture is more transversely stretched than other *Lionepha* (Fig. 17A, B), yielding a notable iridescence, especially in the males. Elytra with at least the third stria visible beyond the posterior dorsal puncture and the second stria almost reaching the elytral apex. It tends to be slightly darker, with darker legs, than other members of the *L. osculans* group, but some specimens are paler. Aedeagus deep, with a bulbous appearance because of the sinuate ventral surface (Fig. 13A, B).

Additional characteristics: Body length 4.68–5.90 mm. Antennae dark, piceous, although the first antennomere can be dark rufous on the underside. Legs in most specimens rufopiceous, occasionally rufous, darker at the joints. Hind wings full-sized. Chromosomes of male 24 + X (Table 5).

Geographic variation: The six specimens sequenced from Oregon have three unique bases within the 28S gene; this is evident in the 28S tree in Figure 5, where the Oregon specimens form a distinct clade.

Note: Hering (1998) reported on the food consumed by '*Bembidion osculans*' on Knowles Creek in Oregon; we examined a selection of specimens from his study, and the majority belong to *Lionepha tuulukwa*, with a few specimens belonging to *L. osculans*

Distribution: A widespread species, common in the Sierra Nevada and coastal areas of California, north to Washington and Idaho (Fig. 24A). As noted by Erwin & Kavanaugh (1981), the record from the Olympic Peninsula in Washington is somewhat doubtful. Commonly encountered between 0 and 2000 m, with a few specimens found up to 2400 m. Found in all months of the year except February; most common in the middle of summer.

Habitat: By far the commonest large *Lionepha*, found in a variety of habitats associated with water, including along the shores of creeks, especially in forests and on the edge of melting snowfields in open conifer forests in the Sierra Nevada.

LIONEPHA SEQUOIAE (LINDROTH, 1963)

(FIGS 3B, 17C, D, 13C, D, 19B, 24B)

Bembidion sequoiae Lindroth, 1963: 260. Holotype ♂ in MCZ (type # 32532), examined. Type locality: Sequoia National Park, California.

Diagnosis: The most distinctive external feature of this moderately large *Lionepha* is the exceptionally small basal protarsomeres in males (Fig. 19B), which are only slightly wider than the second protarsomeres, much smaller than in other *Lionepha* (see other images in Fig. 19). Elytra not iridescent, as sculpticells are not sufficiently transverse (Fig. 17C, D). Aedeagus with broad apex, and with clearly visible dark scales on the internal sac membranes, yielding a speckled appearance (Fig. 13C, D).

Additional characteristics: Body length 4.31–4.95 mm. Antenna piceous, with first antennomere rufous, at least on the underside. Legs rufous, with darker joints. Hind wings full-sized. Chromosomes of male 24 + X (Table 5).

Distribution: This species has been found in the Sierra Nevada of California, the Cascades of Oregon and north to Vancouver, British Columbia (Fig. 24B). There are, in addition, two low-elevation records from coastal California (Sonoma County); although one of these records (from 2.5 miles N of Cazadero on King Ridge, Big Austin Creek; CAS) is relatively recent, both records are unexpected and should be confirmed. Found from 30 to 2230 m in elevation. Specimens were collected from March through September.

Habitat: Found along the gravel and cobble shores of creeks in forests or in forest clearings. Over 80 specimens were found along partly shaded areas of School Creek in the Cascades of Oregon, with most found at the gravel and cobble edge of a splash pool below a culvert.

LIONEPHA PSEUDOERASA (LINDROTH, 1963)

(FIGS 3C, 17E, F, 13E, F, 19C, 24C)

Bembidion pseudoerasum Lindroth, 1963: 260. Holotype ♂ in USNM (type # 76638), examined. Type locality: Truckee, Nevada County, California.

Diagnosis: This species is most easily distinguished externally by the reduced elytral striation, with third stria not visible beyond the posterior dorsal puncture. Elytra only slightly iridescent. Prothoracic sides moderately rounded, in most specimens with

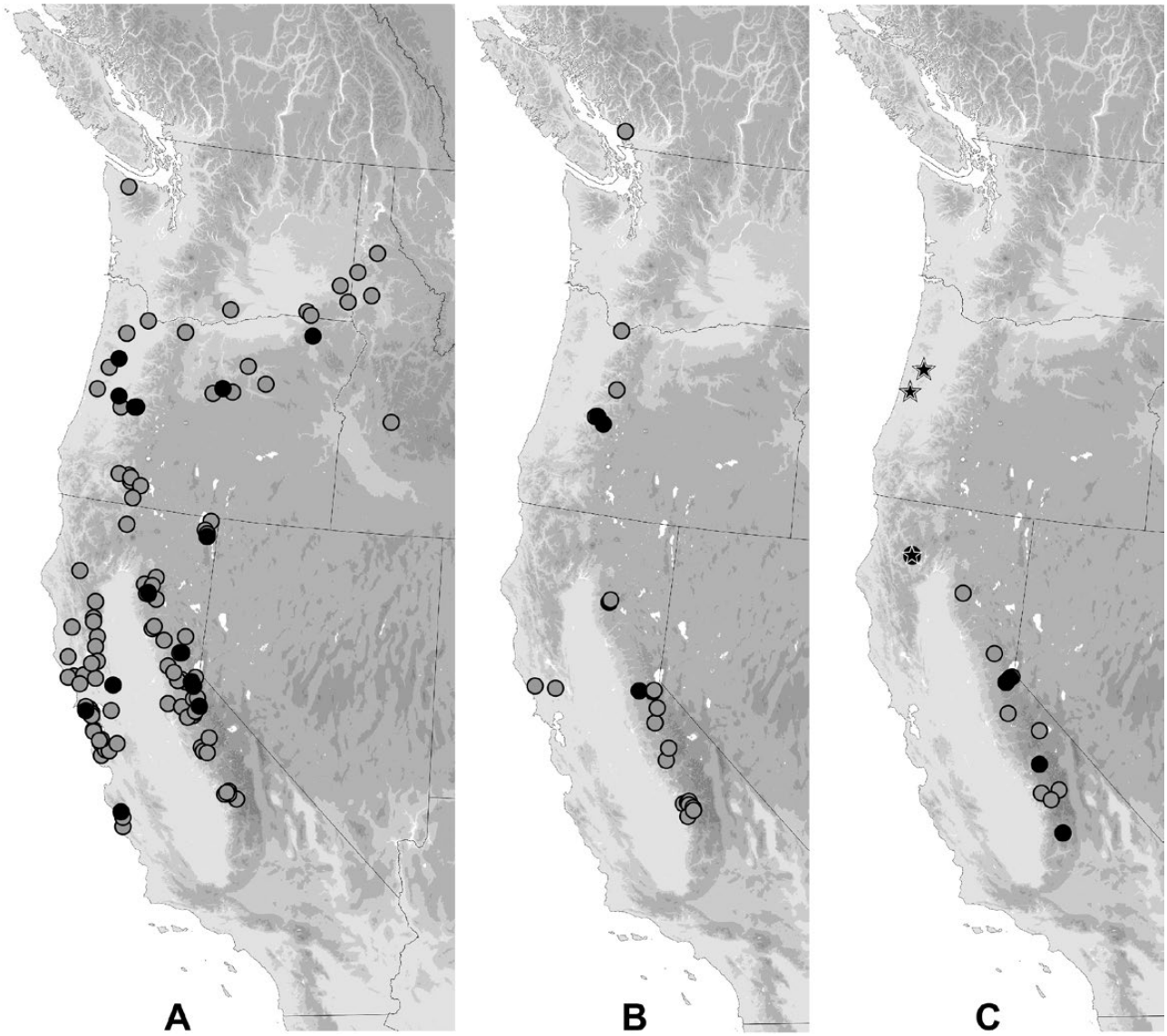

Figure 24. Geographic distribution of members of the *Lionepha osculans* species group. A, *L. osculans*; B, *L. sequoiae*; C, *L. pseudoerasa* (circles) and *L. tuulukwa* (stars). Specimens whose DNA was sequenced are shown in black.

the posterior portion being almost parallel sided (Fig. 3C). Basal protarsomeres of male large, significantly wider than the second tarsomere (Fig. 19C). Aedeagus with ventral surface gently curved (Fig. 11E, F), with internal sac sclerites reduced (although not as much so as in *L. tuulukwa*).

In comparison to *L. tuulukwa*, the prothorax is relatively wider compared to the head (Fig. 3C), and the elytral microsculpture is more transverse (Fig. 17E, F). In comparison to *L. osculans*, narrower and with a less rounded prothorax, and with less iridescent elytra.

Additional characteristics: Body length 4.41–5.25 mm, with most specimens >4.6 mm. Antenna piceous, with underside of first antennomere rufous. Legs rufous or dark rufous, with darker joints. Hind wings full-sized. Male with 25 chromosomes (Table 5).

Distribution: This species is only known from the Sierra Nevada and Trinity Alps of California (Fig. 24C). A mid-elevation species, found from 1200 to 2720 m, from May through August.

Habitat: This rarely collected species has been found on sand and silt near streams and ponds in forests.

LIONEPHA TUULUKWA MADDISON, SP. NOV.

(FIGS 3D, 17G, H, 13G, H, 19D, 24C)

http://zoobank.org/urn:lsid:zoobank.org:act:BC118CC8-0693-4497-87F8-167098DC6EF4

Holotype ♂ (OSAC), herein designated, labelled: 'USA: Oregon: Benton Co., Marys Peak Rd, Alder Creek Falls, 700 m, 44.4746°N 123.5286°W, 24 September 2010. DRM 10.133. D.R. Maddison', 'David R. Maddison DNA2643 DNA Voucher' [pale green paper], 'HOLOTYPE Lionepha tuulukwa David R. Maddison' [partly handwritten, on red paper], 'Oregon State Arthropod Collection OSAC_0002000006 [matrix code]' [printed on both sides of white paper]. Genitalia mounted in Euparal in between coverslips pinned with specimen; extracted DNA and chromosome slide stored separately. GenBank accession numbers for DNA sequences of the holotype are MN401816, MN401919, MN401967, MN402036, MN402162, MN402273 and MN402355.

Paratypes (70): In addition to 33 paratypes from the type locality (deposited in OSAC, CAS, CNC, CMNH, USNM, MCZ, MNHN, MZLU, NHMUK, UASM), we designate 37 paratypes from the following localities: USA: Oregon: Benton Co., Marys Peak, Alder Creek Falls, 700 m, 44.4745°N 123.5282°W (1, OSAC); USA: Oregon: Benton Co., Marys Peak Rd, nr Alder Creek Falls, 700 m, 44.4748°N 123.5280°W (21, OSAC); USA: Oregon: Benton Co., Waterfall at mile 2.2 Marys Peak Rd, 700 m, 44.4748°N 123.5247°W (5, OSAC); USA: Oregon: Lane Co., Knowles Creek, 6.8 km SE Mapleton, 48 m, 44.0136°N 123.7880°W (7, OSAC); USA: Oregon: Lane Co., Knowles Creek (3, OSAC).

The single specimen from the Trinity Alps of California (USA: California: Trinity Co., Canyon Creek, 1440 m, 40.9490°N 123.0179°W; OSAC) was not designated a paratype.

Type locality: The type locality is Alder Creek Falls, on the south-east slope of Marys Peak, in the Oregon Coast Range, at 44.4746°N 123.5286°W (Fig. 4A).

Etymology: The epithet is derived from *tuu-lukwa*, meaning 'waterfall' in the Santiam dialect of Kalapuya (Swadesh, 1965), and it is thus most likely the same word in the Marys River dialect (Henry Zenk, pers. comm., 2013). In addition to describing the habitat of these beetles at the type locality, the name also honours the first people of Marys Peak, who knew these lands so well.

Diagnosis: Within the *L. osculans* group it is distinguished by the less transversely stretched meshes on the elytra; in males, most sculpticells are about three times as wide as tall (Fig. 17G); in females, they are brick-like and deeply engraved (Fig. 17H). Because of the exceptionally dull lustre of their elytra, females are among the easiest *Lionepha* to identify to species. In contrast, the elytral microsculpture is more transverse than any of the smaller *Lionepha* (*erasa* group). Prothorax narrow, only slightly wider than the large head (Fig. 3D). Basal protarsomeres of male large, significantly wider than the second tarsomere (Fig. 19D). Aedeagus (Fig. 13G, H) most similar to that of *L. pseudoerasa*, but with even more reduced internal sac sclerites.

Additional characteristics: Body length 4.26–5.07 mm, with most specimens >4.5 mm. Surface of elytra in many specimens with a slight metallic reflection, brassy or greenish in colour. Antennae dark, piceous, except for base of first antennomere, which can be dark rufous on the underside. Legs rufous or rufopiceous, darker at the joints. Hind wings full-sized. Chromosomes of male 24 + X (Pflug *et al.* in review).

Geographic variation: The single specimen from the Trinity Alps of California (DNA4113) is distinctive in both 28S and *COI*. In 28S, the California specimen has three insertions not present in Oregon *L. tuulukwa*, totalling six bases; it also has one five-base deletion relative to the Oregon specimens. In *COI*, this same specimen has a triplet that codes for valine (at position 305 within its sequence in GenBank, accession MN402204), as do all *Lionepha* from other species, whereas the Oregon *L. tuulukwa* have the derived state of isoleucine at that position.

Note: Referred to as *Lionepha* 'Waterfalls' in Sproul & Maddison (2017) and Pflug *et al.* (in review). See also the note under *Lionepha osculans*.

Distribution: This species is currently known from only three localities: two in the Oregon Coast Range and one in the Trinity Alps of California (Fig. 24C). The Coast Range localities are 50 and 700 m in elevation, with the Trinity Alps locality at 1440 m. Specimens have been found in April, June, August and September. At Alder Creek Falls, they are most commonly encountered in late August and September, after the first autumn rains.

Habitat: At the type locality, found in the splash zone of Alder Creek Falls (Fig. 4A), and on nearby seeps running down large rockfaces. They crawl among wet moss and soil in these areas, and are most easily found at night. At the other two known localities (Knowles Creek west of Eugene, Oregon, and Canyon Creek in the Trinity Alps of California) they were found under

rocks on the gravel and cobble shores of small creeks in forests (Fig. 4B).

CONCLUSIONS

Although we are confident of the 11 species as distinct evolutionary lineages within *Lionepha*, there are some populations that require further study. The separation between the most doubtful pair, *L. erasa* and *L. australerasa*, should be tested by a search for intervening populations in the Oregon Cascades. In addition, there are a few populations that are distinctive enough morphologically or molecularly to warrant further study: the Utah populations of *L. probata*, the populations of *L. erasa* from south-central British Columbia, the central Washington populations of *L. casta*, *L. kavanaughi* from the Bitterroots and the California populations of *L. tuulukwa*. It is possible there are more species to be recognized.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the publisher's web-site.

File S1. Complete data matrix: S1.AlignmentsAndIQTreeAnalyses.nex.
Table S1. Localities of capture of *Lionepha* specimens that were sequenced. Four-digit numbers at the start of each row are D. R. Maddison DNA voucher numbers. For specimens that are not housed in OSAC, specimen numbers for the repository are given in Supporting Information, Table S2.
Table S2. Voucher codes for those vouchers not housed in OSAC. Four-digit numbers in the '#' column are D. R. Maddison DNA voucher numbers.
Figure S1. Maximum likelihood tree for the concatenated, eight-gene matrix. Branch length is shown proportional to relative divergence, as estimated by IQ-TREE; scale bar indicate 0.01 units. Outgroups not depicted.
Figure S2. Part of the D2 expansion region of 28S, showing variation in insertion and deletions across *Lionepha*. Each species has a unique set of insertions and deletions. For all species except *Lionepha disjuncta*, only a sampling of sequenced specimens is shown.