

A new cryptic *Trypophloeus* Fairmaire, 1864 species in Northern Fennoscandia (Coleoptera, Curculionidae) revealed by DNA analyses

TORSTEIN KVAMME, MICHAEL MANDELSHTAM, MARIA SALNITSKA, DARIO I. OJEDA & ÅKE LINDELÖW

Kvamme, T., Mandelshtam, M., Salnitska, M., Ojeda, D. I. & Lindelöw, Å. 2021. A new cryptic *Trypophloeus* Fairmaire, 1864 species in Northern Fennoscandia (Coleoptera, Curculionidae) revealed by DNA analyses. *Norwegian Journal of Entomology* 68, 44–66.

Specimens of the genus *Trypophloeus* Fairmaire, 1864 collected in Fennoscandia from *Salix myrsinifolia* Salisb. and *S. glauca* L. were studied both morphologically as well as with phylogenetic analyses based on DNA sequences of the COI and 28S rDNA. Based on the results a new species is described and named *Trypophloeus borealis* **sp. n.** Scandinavian specimens of this new species were previously misidentified as *Trypophloeus dejevi* Stark, 1936. The phylogenetic and sequence divergence analyses based on these gene regions show that *Trypophloeus nitidus* Swaine, 1912, in North America is the closest related species. Information on the biology and known distribution is included. The new species is a member of the *Trypophloeus alni* (Lindemann, 1875) species-group. The *T. alni* species-group is well demarcated and supported by the DNA results. The rest of the species in the genus *Trypophloeus* are proposed to be divided into three other species groups.

A classic taxonomic study on the species in the *Trypophloeus alni* species-group was also carried out. In general, the morphology of the genus gives a poor basis for taxonomy and morphological identification due to small differences and overlapping characters between the species. Still, the characters can give some basis for identification when a large material is examined. However, the overlap of morphological characters makes the identification uncertain. It should be interpreted as an indication more than an exact identification, even when combined with geographical, biological and host tree information. The conclusion is that *T. borealis* **sp. n.** and *T. nitidus* are morphologically very similar with overlapping characters and are morphological sibling species. Biometric and statistical studies might bring more information on morphological differences.

The presented study caused some nomenclatorial changes. *Trypophloeus niger* Stark, 1936 **syn. n.** is a synonym of *Trypophloeus dejevi*. The type of *Trypophloeus striatulus* (Mannerheim, 1853) was considered lost, but has been found in the collection of the Zoological Institute in St. Petersburg, Russia. Many authors erroneously considered the species to belong to the genus *Trypophloeus*. However, studies of the type show that it actually belongs to the genus *Cryphalus* Erichson, 1836. *Cryphalus striatulus* Mannerheim, 1853 has priority, and the name is resurrected and *Cryphalus ruficollis* Hopkins, 1915 **syn. n.** is thus a new junior synonym. Consequently, *Trypophloeus striatulus* (Mannerheim, 1853) is not a valid name and *Trypophloeus nitidus* Swaine, 1912 **res. n.** is the valid name.

Information on the biology and known distribution of *T. borealis* **sp. n.** is included. Lectotype for *Cryphalus alni* Lindemann, 1875 (now called *Trypophloeus alni*) is selected from Lindemann's specimens preserved in Natural History Museum in Vienna and is designated here.

Key words: Coleoptera, Curculionidae, Scolytinae, Trypophloeini, *Trypophloeus*, taxonomy, nomenclature, biology, new species, new synonym, new resurrection, *Cryphalus*, Cryphalini.

Torstein Kvamme, Norwegian Institute of Bioeconomy Research, P. O. Box 115, NO-1431 Ås, Norway, E-mail: Torstein.Kvamme@nibio.no

Michail Mandelshtam, St. Petersburg State Forest Technical University named after S.M. Kirov, Department of Forest Protection, Wood Science and Game Management, Institutskii per., 5, St. Petersburg, 194021, Russia. E-mail: amitinus@mail.ru

Maria Salnitska, University of Tyumen, X-BIO Institute, 6 Volodarskogo Str., Tyumen, Russia. E-mail: m.salnickaya@utmn.ru

Dario I. Ojeda, Norwegian Institute of Bioeconomy Research, P. O. Box 115, NO-1431 Ås, Norway, E-mail: dario.alayon@nibio.no

Åke Lindelöw, Swedish university of agricultural sciences, Department of Ecology, P.O. Box 7044, SE-750 07 Uppsala, Sweden. E-mail: ake.lindelow@slu.se

Introduction

The tribe Cryphalini was recently revised and divided into four tribes (Johnson *et al.* 2020). As a result, the genus *Trypophloeus* Fairmaire, 1864 was allocated to the tribe Trypophloeini.

The genus *Trypophloeus*, includes pygmy borers with 5-segmented antennal funiculus and elongated fusiform club. The species are small, between 1.2–2.3 mm (*cf.* Wood 1982, Pfeffer 1995), and have few distinct external morphological characters. In addition, the aedeagus have few useful structures, which also complicates the separation of the species. As a result, misidentifications have often occurred, and the genus is strongly in need of a revision. The scientific names in different publications are not used in a uniform manner and reflect the taxonomic and nomenclatorial confusion in the genus.

Trypophloeus species infest host trees of three principal genera: *Populus* spp. and *Salix* spp. (family Salicaceae), and *Alnus* spp. (family Betulaceae). The species are monogamous, forming a simple cave-like breeding chamber where eggs are laid in batches. Larvae feed individually in the phloem and maturation feeding by young adults take place in the bark of living plants.

The genus *Trypophloeus* has a Holarctic distribution and according to Wood (1982) there are four species known from the Nearctic region. Twelve species are listed from the Palaearctic region (Knížek 2011). Johnson *et al.* (2020)

mentioned that the genus, in total has 15 species, but do not present a complete species list.

The use of species names in Fennoscandia is inconsistent when comparing the different sources. Five species of the genus *Trypophloeus* are listed from Norway by Kvamme & Lindelöw (2014), including *T. borealis* **sp. n.** under the name *T. dejevi* Stark, 1936. Silfverberg (2010) listed five species and two subspecies, while Knížek (2011) mentioned six species from Fennoscandia including *T. granulatus* (Ratzeburg, 1837). No records of *T. granulatus* in Scandinavia are known and we thus question if this is correct.

Due to their small size and hidden life style, combined with little economic impact, the species within *Trypophloeus* are overlooked and have paid little attention by forest entomologists. However, *Trypophloeus populi* Hopkins, 1915 is known to transfer aspen canker causing sudden aspen decline (SAD) in Western United States and *T. nitidus* Swaine, 1912 killing large areas of *Salix alaxensis* (Anderson) in Canada (Furniss 2004). Therefore, Trypophloeini deserve more attention both from taxonomists and ecologists (Marchetti *et al.* 2011, Furniss 2004).

Lindelöw (2009) published *T. dejevi* as a new species from Europe. One specimen was collected in 1969 by the late Lars Huggert. The locality, Torne Lappmark: Årosjokk (67°52' N/19°22'E), Northern Sweden. In 2008, the same locality was visited, and the species was rediscovered in *Salix myrsinifolia* (*cf.* Lindelöw 2009).

Miloš Knížek and Michail Mandelshtam first

identified the specimen as *T. dejevi*. However, the exact species assignment was questionable. The closest known area where *T. dejevi* occurs, is the Sajon Mountains (East Siberia), more than five thousand kilometers east from the collecting site in Northern Sweden. Later the species was also recorded in Norway and Finland (Lindelöw & Kvamme 2013). So far, all the Fennoscandian records are from areas north of the Arctic Circle. Due to the uncertainty about of the species status, a taxonomic study combined with DNA analyses was carried out. Based on these analyses we propose and describe a new species, *Trypophloeus borealis* **sp. n.**

Material and methods

Specimens studied. The specimens included in the DNA study are listed under each species (Table 1).

Morphological and taxonomic studies are based on specimens listed for each species under the heading “Descriptions and taxonomy”. The present study included specimens from the collections listed below. Abbreviations used in the text are written in bold: **APP** = Alexander A. Petrov private collection (Moscow, Russia). **BEM** = W.F. Barr Entomological Museum (Moscow, Idaho, USA) (Curator Frank W. Merickel). **CNCI** = Canadian National Collection of Insects and Nematodes (Ottawa, Canada) (Curator Patrice Bouchard). **CTK** = Torstein Kvamme private collection (Ås, Norway). **CÅL** = Åke Lindelöw private collection (Uppsala, Sweden). **IBSS** = Institute of Biology and Soil Sciences (Vladivostok, Russia). **ISEA** = Siberian Zoological Museum, Institute for Systematics and Ecology of Animals (Novosibirsk, Russia). **NHMW** = National History Museum in Vienna (Vienna, Austria). **NHRS** = Swedish Museum of Natural History (Stockholm, Sweden). **SEM** = Snow Entomological Museum (Lawrence, Kansas, USA). **USNM** = US National Museum (Washington, USA (**USNM**)) (Curator Natalia Vandenberg). **ZIN** = Zoological Institute (St. Petersburg, Russia). **ZMMU** = Zoological Museum of Moscow State University, including

V. Grachev and N. Nikitsky collections (Moscow, Russia).

Taxon sampling and gene regions sequenced.

We included DNA sequences of 14 out of the 15 currently recognized species within *Trypophloeus* (*cf.* Johnson *et al.* 2020). In this study we generated new sequences of COI and 28S rDNA for 10 specimens representing four species within this genus. The analyses were complemented with sequences of these two genes from GenBank, the Barcode of Life Data System (www.barcodinglife.org) (Ratnasingham & Hebert 2007), and from a previous phylogenomic analyses in this group (Johnson *et al.* 2018) (Table 1).

DNA extraction and sequencing. Before DNA extraction, the specimens were washed in 96% ethanol and dried at 50° C for 50 minutes. DNA was extracted from ethanol-fixed or dried pinned beetles using the DNeasy Plant Mini Kit (Qiagen) according to the manufacturer’s instructions. Amplification was performed in 50 µl containing 1x PCR Buffer (Qiagen) supplemented with 1 mM MgCl₂, 0.2 mM of each dNTP, 0.2 µM of each primer, 2 U HotStar Taq Plus (Qiagen) and 1 µl DNA template using a GeneAmp PCR System 9700 (LifeTechnologies). COI and 28S rDNA were amplified with the primer sequences S1718, A2237, S3690 and A4285 following Jordal *et al.* (2011) and with primer A4396 (5’-TGCGGTGGTATCGACAAGCGT-3’). The following cycling conditions were used: 5 min initial activation step at 94° C followed by 3-step cycling of 94° C, 30 sec, with 46° C annealing temperature, 50 sec (COI) or 55° C, 60 sec (28S rDNA), 72° C, 1 min for 40 cycles and a final extension at 72° C for 10 min. PCR products were cleaned with Qiaquick PCR Cleanup Kit (Qiagen) before sequencing. Sequences were inspected and trimmed with Geneious prime 2020. Sequences for COI and 28S rDNA were deposited to GenBank under BioProject PRJNA634471 (Table 1).

Alignment, phylogenetic analyses and sequence divergence estimation. Each matrix was aligned with AliView (Larsson 2014) and summary statistics were obtained with AMAS (Borowiec

2016). Each matrix was analyzed with maximum likelihood (ML) with IQ-TREE (Nguyen *et al.* 2015) using the best model for each region gene and 200 bootstrap replicates. We also performed a Bayesian analysis as implemented in MrBayes 3.2.6 (Huelsenbeck & Ronquist 2001, Ronquist *et al.* 2012) using four chains, two runs of 20 million generations with the GTR+ Γ model of nucleotide substitution, the invgamma rate of variation and a sample frequency of 1000. The performance of the Bayesian analysis was assessed with Tracer 1.7 (Rambaut *et al.* 2018) with effective sample sizes (ESS) higher than 200 for all parameters. ML and Bayesian analyses were performed separately on each gene region. Our analyses with COI included 59 specimens representing 14 out of the 15 species currently recognized in *Trypophloeus*, while the 28S rDNA matrix included 21 specimens representing 10 species. In the COI analyses we used three species of *Hypothenemus* as an outgroup, which is one of the closest genera to *Trypophloeus* according to the most recent phylogenomic analyses (Johnson *et al.* 2020, 2018). In the 28S rDNA analyses we used *T. populi* as an outgroup. In addition, we also performed ML and Bayesian analyses on the combined sequences of COI and 28S rDNA using the above-mentioned parameters; however, we only used the specimens sequenced in this study and a representative specimen for *T. bispinulus*, *T. tremulae*, *T. granulatus grothi*, *T. populi* and *T. salicis*. The corresponding sequences of these five species were selected from the individual phylogenetic analyses of each gene, and the two sequences (COI and 28S rDNA) do not correspond to the same specimen, but rather represent each species we recovered as a monophyletic lineage in the COI analyses. Sequences were concatenated with AMAS and aligned with AliView. Sequence divergence among the concatenated sequences of COI and 28S rDNA were estimated with the MEGA6 software package (Tamura *et al.* 2013). All trees were visualized and edited using FigTree v1.4.3 (Rambaut 2016).

The length of 50 specimens of *T. borealis* **sp. n.** imagines, glued on paper cards, were measured using a stereo microscope Leica MS5 with an ocular micrometer. The length was defined as the

distance from the anterior edge of pronotum to the posterior tip of elytra. The measurements are given in mm with an accuracy of 0.01 mm. The measurements of the other species are based on the measurements in the presented manuscript and Wood (1982).

Nomenclature

Unless otherwise stated, the nomenclature of the Palaearctic species used here follows Knížek (2011) and the nomenclature of the Nearctic species follows Wood (1982). At the tribe level we follow Johnson *et al.* (2020) and use the name Trypophloeini. We use the name *T. nitidus* Swaine, 1912 **res. n.** instead of *T. striatulus* auct., nec Mannerheim, 1853 (*cf.* page 23 and 30).

The names following the sequences generated from databanks and other sources are kept unchanged, although they are not updated and do not necessary reflect the most current nomenclature.

Results

Individual phylogenetic analyses based on COI and 28S rDNA. The alignment matrix of COI consisted of 521 bp with 197 parsimony informative (PI) sites (37%). In contrast, we found lower levels of variation in 28S rDNA with only 89 PI (12.6%) in the aligned matrix (705 bp). We recovered similar topologies using both gene regions with ML and Bayesian analyses with moderate to high support for most branches (Figure 1 and 2). Our results based on COI recovered all members of the *T. alni* species-group nested together in a monophyletic lineage with *T. granulatus* as their sister taxa (Figure 1). Our analyses suggest that all Nearctic species are grouped into two clades, one monophyletic lineage containing *T. salicis*, *T. populi* and *T. thatcheri*, while *T. nitidus* was recovered as the sister species to *T. borealis* **sp. n.**, nested with other species of the *T. alni* group (Figure 1). We recovered a similar relationship among members of the *T. alni* species-group with the 28S rDNA,

TABLE 1. List of specimens used in the phylogenetic analyses. Accessions from GenBank, the Barcode of Life Data System (BOLD), and references from previous studies is provided. Newly sequences generated in this study are also listed.

Species name	Location and host	Voucher	GenBank and Barcode of Life (BOLD) accessions		Reference
			COI	28S rDNA	
Outgroup					
<i>Hypothenemus hampei</i>	USA, Puerto Rico	UPR13Hypham	MK622712	-	Vega et al. 2020
<i>Hypothenemus seriatus</i>	USA, Puerto Rico	UPR66Hypseriatus	MK622767	-	Vega et al. 2020
<i>Hypothenemus obscurus</i>	USA, Puerto Rico	UPR58Hypobsc	MK622758	-	Vega et al. 2020
<i>Trypophloeus asperatus</i> (= <i>Cryphalus asperatus</i>)	Ukraine, Carpathians	10064509	KU144883	-	Mandelsham et al. Unpub.
	Ukraine, Carpathians	10064538	KU144884	-	Mandelsham et al. Unpub.
	UK	BMNH 1046450	NC_036285	-	Miller et al. Unpub.
	UK	BMNH 1046450	KX035204	-	Miller et al. Unpub.
Ingroup					
<i>Trypophloeus alni</i>	Russia, Orzhitsy, Lomonosov district, Leningrad Prov., 9.V.2001, host <i>Alnus incana</i> , Leg et det. M. Mandelsham. Ethanol preserved	No. 10	MT672002	MW016385	This study
	Russia	CrTry01	JX263805	-	Jordal & Cognato 2012
	Russia, Leningrad Prov, Orzhitsy	CrTry01	KU144900	-	Mandelsham et al. Unpub.
<i>Trypophloeus asperatus</i> (= <i>T. binodulus</i>)	Germany, Thuringia, Erfurt, Noeda, Auwald	ZFMK-TIS-2535593	KU913568	-	Rulik and Ahrens Unpub.
	Norway, More og Romsdal	BHJ316	-	SCOL334-12	Stenberg Unpub.
	Norway, More og Romsdal	BHJ315	-	SCOL302-12	Stenberg Unpub.
	Norway, More og Romsdal	BHJ318	-	SCOL304-12	Stenberg Unpub.
	Norway, More og Romsdal	BHJ320	-	SCOL335-12	Stenberg Unpub.
<i>Trypophloeus binodulus</i>	Norway, More og Romsdal, Nesset	BHJ315	KU144911	-	Mandelsham et al. Unpub.
	Norway, More og Romsdal, Nesset	BHJ318	KU144910	-	Mandelsham et al. Unpub.
<i>Trypophloeus bispinulus</i>	Norway, More og Romsdal, Nesset	BHJ317	KU144912	-	Mandelsham et al. Unpub.
	Finland, northern Ostrobothnia, Kiiminki	ZMUO/FIN:003978	KJ965579	-	Pentisaari et al. 2014
	Russia, Leningrad Prov, Lomonosov, Old Peterhof	GBCL49834-19	KU144913	-	Mandelsham et al. Unpub.

TABLE 1. continued.

Species name	Location and host	Voucher	GenBank and Barcode of Life (BOLD) accessions		Reference
			COI	28S rDNA	
<i>Trypophloeus bispinulus</i>	Russia, Leningrad Prov, Kuznechnoye	10064516	KU144892	-	Mandelshtam <i>et al.</i> Unpub.
	Russia, Leningrad Prov, Kuznechnoye	10064523	KU144891	-	Mandelshtam <i>et al.</i> Unpub.
	Russia	CrTry02	KC845440	-	Jordal & Kambestad 2014
	Russia, Pskov Prov, Astratovo	10064525	KU144894	-	Mandelshtam <i>et al.</i> Unpub.
	Russia, Pskov Prov, Astratovo	10064514	KU144893	-	Mandelshtam <i>et al.</i> Unpub.
	Norway	BHJ317	SCOL303-12	-	Jordal Unpub.
	Russia, Saint Petersburg	BHJ225	SCOL214-12	SCOL214-12	Mandelshtam <i>et al.</i> Unpub.
<i>Trypophloeus borealis</i> sp. n.	Norway, Fi Karasjok, Jergul (EIS 166) 3.VII.2012 host <i>Salix myrsinifolia</i> Leg. et det. Å. Lindelöw. Ethanol conserved	No. 12	MT672003	MW016383	This study
		No.12B	MT672007	MW016384	This study
<i>Trypophloeus dejevi</i>	Russia, Vladivostok, 16.VIII.2000, host: <i>Populus tremula</i> . Leg et det. M. Mandelshtam. Dry mounted	No. 7	MT672000	MW016386	This study
		No. 8	MT672001	MW016387	This study
		No. 9	MT672006		This study
	Russia, Primorsky Krai, Arseniev	BHJ283	KU144914	-	Mandelshtam <i>et al.</i> Unpub.
	Russia, Primorsky Krai, Anisimovka	CrTry09_4	KU144903	-	Mandelshtam <i>et al.</i> Unpub.
	Russia, Primorsky Krai, Anisimovka	CrTry09_3	KU144902	-	Mandelshtam <i>et al.</i> Unpub.
	Russia	AJJ_64	MG051184	-	Johnson <i>et al.</i> Unpub.
	Russia, Primorskiy Kray	BHJ279	SCOL266-12	-	Jordal Unpub.
	Russia	UFIFAS_UFFE_13677	-	MT120980	Johnson <i>et al.</i> 2018
<i>Trypophloeus discedens</i>	Ukraine, Kharkov Prov, Gaidary	10064510	KU144885	-	Mandelshtam <i>et al.</i> Unpub.
	Sweden, Up Fiby	CrTry08	KU144904	-	Mandelshtam <i>et al.</i> Unpub.
	Russia, Yaroslavl Prov, st Molot	10064512	KU144895	-	Mandelshtam <i>et al.</i> Unpub.
	Ukraine, Kharkov Prov, Gaidary	10064519	KU144886	-	Mandelshtam <i>et al.</i> Unpub.

TABLE 1. continued.

Species name	Location and host	Voucher	GenBank and Barcode of Life (BOLD) accessions		Reference
			COI	28S rDNA	
<i>Trypophloeus granulatus</i>	Norway, Aust-Agder	BHJ56	SCOL054-12	SCOL054-12	Jordal Unpub.
	Ukraine, Carpathians	CrTry05_2	KU144906	-	Mandelshtam et al. Unpub.
	Norway, More og Romsdal	BHJ319	SCOL305-12	SCOL305-12	Stenberg Unpub.
	Ukraine, Carpathians	10064536	KU144889	-	Mandelshtam et al. Unpub.
	Ukraine, Carpathians	10064529	KU144890	-	Mandelshtam et al. Unpub.
	Ukraine, Carpathians	CrTry05_1	KU144905	-	Mandelshtam et al. Unpub.
	Norway, More og Romsdal	BHJ321	-	SCOL336-12	Stenberg Unpub.
<i>Trypophloeus klimeschi</i>	Tajikistan, Pamirs, Gishun	10064503	KU144898	-	Mandelshtam et al. Unpub.
	Tajikistan, Pamirs, Gishun	10064502	KU144899	-	Mandelshtam et al. Unpub.
<i>Trypophloeus nitidus</i>	USA, Alaska. 1996. <i>Salix alaxensis</i> . Leg. et det. M.M.Furniss. Dry mounted	No. 14	MT672004	MW016382	This study
		No. 14B	MT672005	MW016380	This study
		No. 14C	MT672008	-	This study
		No. 14D	MT672009	MW016381	This study
<i>Trypophloeus populi</i>	USA	CrTry03_2	KU144909	-	Mandelshtam et al. Unpub.
	USA	CrTry03	KC845441	-	Jordal & Kambestad, 2014
	USA	UFIFAS_UFFE_14156	-	MT120929	Johnson et al. 2018
<i>Trypophloeus rybinski</i>	Ukraine, Crimea, Demerdzhi	CrTry06_1	KU144907	-	Mandelshtam et al. Unpub.
<i>Trypophloeus salicis</i>	USA	I10239	-	-	Johnson et al. 2018
		UFIFAS_UFFE_13951	-	MT120902	Johnson et al. 2018
<i>Trypophloeus striatulus</i>	USA, Alaska, Chandalar Shelf	UAM: Ento:111009	KU876418	-	Sikes et al. 2017
	USA, Alaska, Chandalar Shelf	UAM: Ento:111008	KU876419	-	Sikes et al. 2017
<i>Trypophloeus thatcheri</i>	USA	10064484	KU144897	-	Mandelshtam et al. Unpub.
	USA	10064482	KU144896	-	Mandelshtam et al. Unpub.

TABLE 1. *continued.*

Species name	Location and host	Voucher	GenBank and Barcode of Life (BOLD) accessions		Reference
			COI	28S rDNA	
<i>Trypophloeus tremulae</i>	Ukraine	CrTry04	KC845442	-	Jordal & Kambestad, 2014
	Ukraine, Crimea, Magabi	CrTry04_1	KU144908	-	Mandelshtam <i>et al.</i> Unpub.
	Russia, Krasnodar	10064500	KU144887	-	Mandelshtam <i>et al.</i> Unpub.

TABLE 2. Distance matrix of the specimens sequenced in this study with the COI gene.

	<i>T. alni</i>	<i>T. dejevi</i> no. 7	<i>T. dejevi</i> no. 9	<i>T. dejevi</i> no. 8	<i>T. borealis</i> no. 12	<i>T. borealis</i> no. 12B	<i>T. nitidus</i> no. 14A	<i>T. nitidus</i> no. 14B	<i>T. nitidus</i> no. 14C	<i>T. nitidus</i> no. 14D
<i>T. alni</i>		91.4	91.4	91.3	90.5	90.5	89.6	89.6	89.6	89.6
<i>T. dejevi</i> no. 7	91.4		100	99.8	90.1	91.1	89.6	89.6	89.6	89.6
<i>T. dejevi</i> no. 9	91.4	100		99.8	90.1	90.1	89.6	89.6	89.6	89.6
<i>T. dejevi</i> no. 8	91.3	99.8	99.8		90	90	89.4	89.4	89.4	89.4
<i>T. borealis</i> no. 12	90.5	90.1	90.1	90		100	97.6	97.6	97.6	97.6
<i>T. borealis</i> no. 12B	90.5	90.1	90.1	90	100		97.6	97.6	97.6	97.6
<i>T. nitidus</i> no. 14A	89.6	89.6	89.6	89.4	97.6	97.6		100	100	100
<i>T. nitidus</i> no. 14B	89.6	89.6	89.6	89.4	97.6	97.6	100		100	100
<i>T. nitidus</i> no. 14C	89.6	89.6	89.6	89.4	97.6	97.6	100	100		100
<i>T. nitidus</i> no. 14D	89.6	89.6	89.6	89.4	97.6	97.6	100	100	100	

TABLE 3. Distance matrix of the specimens sequenced in this study with the 28S rDNA gene.

	<i>T. alni</i>	<i>T. dejevi</i> no. 7	<i>T. dejevi</i> no. 8	<i>T. nitidus</i> no. 14A	<i>T. nitidus</i> no. 14D	<i>T. nitidus</i> no. 14B	<i>T. borealis</i> no. 12	<i>T. borealis</i> no. 12B
<i>T. alni</i>		99	99	97.3	97.3	97.1	99.6	97
<i>T. dejevi</i> no. 7	99		100	97	97	96.8	99.6	96.6
<i>T. dejevi</i> no. 8	99	100		97	97	96.8	99.6	96.6
<i>T. nitidus</i> no. 14A	97.3	97	97		100	99.8	99.6	99.7
<i>T. nitidus</i> no. 14D	97.3	97	97	100		99.8	99.6	99.7
<i>T. nitidus</i> no. 14B	97.1	96.8	96.8	99.8	99.8		99.6	99.5
<i>T. borealis</i> no. 12	96.9	96.9	96.9	99.6	96.6	99.6		99.8
<i>T. borealis</i> no. 12B	97	96.6	96.6	99.7	99.7	99.5	99.8	

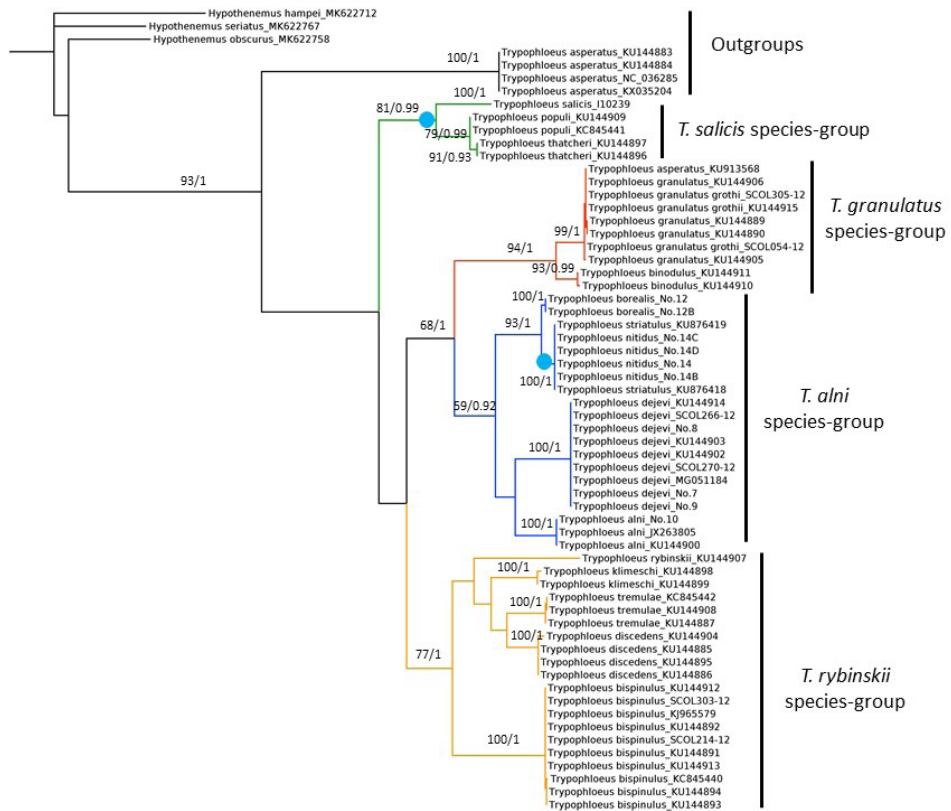


FIGURE 1. Best tree obtained with the phylogenetic reconstruction using COI and ML as implemented in IQ-TREE. Values next to the branches indicate 200 bootstrap support values and posterior probability for the Bayesian analysis. Nearctic species are indicated with a blue dot.

Note: *Trypophloeus asperatus* (Gyllenhal, 1813) is an old wrong name for *T. binodulus* (Ratzeburg, 1837) used in many sources due to misinterpretation of the species name by Pfeffer (1995) and by other old authors. The former name is used here solely to conform old deposited sequencing data files. The outgroup belongs to genus *Cryphalus* despite the genus placement was already justified by Wood (1972) (cf. also Knížek 2011). *Trypophloeus asperatus* on the sequence in the *T. granulatus* group refers to misidentified *T. binodulus* and naming since the DNA sequence group together with *T. granulatus* and not the true *C. asperatus* (Figure 1).

albeit with a lower number of species used in these analyses (Figure 2).

Phylogenetic combined analyses and sequence difference estimates. The combined analyses further support the relationships among the *T. alni* species-group, with the specimens of *T. nitidus* as the sister species of *T. borealis*. Both ML and Bayesian inferences recovered the same topologies with high bootstrap and Bayesian

posterior probabilities (Figure 3). Overall, we found lower pairwise nucleotide differences in 28S rDNA than COI (Tables 2 and 3), similar to previous studies using the same set of markers together (Cognato 2006, Resch et al. 2014). Sequence analyses of COI and 28S rDNA showed lower pairwise nucleotide differences between *T. borealis* and *T. nitidus* (COI = 2.4%, 28S rDNA = 0.38%) than between *T. alni* and *T. deveji* (COI = 8.6%, 28S rDNA = 1.0%).

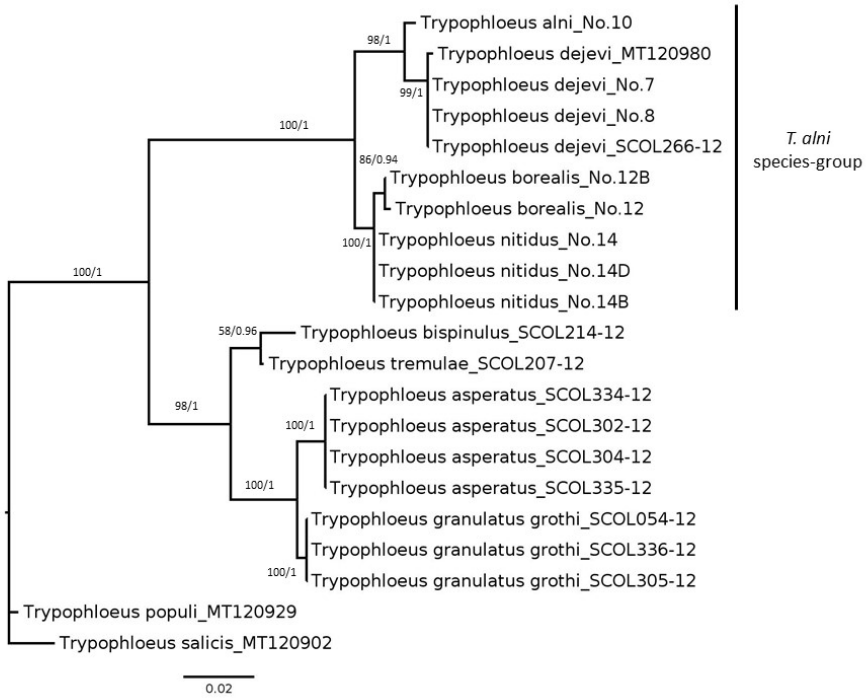


FIGURE 2. Best tree obtained with the phylogenetic reconstruction using 28S rDNA and ML. Values next to the branches indicate 200 bootstrap support values and posterior probability for the Bayesian analysis.

Note: *Trypophloeus asperatus* (Gyllenhal, 1813) is an old wrong name for *T. binodulus* (Ratzeburg, 1837) used in many sources due to misinterpretation of Pfeffer (1995) and by other old authors; the former name is used here solely to conform old deposited sequencing data files, in fact the clade *Trypophloeus asperatus* refers to *T. binodulus*.

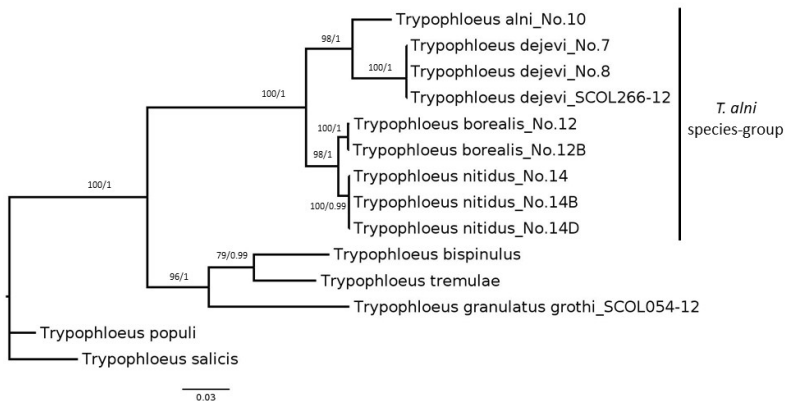


FIGURE 3. Best tree obtained with the phylogenetic reconstruction using the combined sequences of COI and 28S rDNA with ML as implemented in IQ-TREE. Values next to the branches indicate 200 bootstrap support values and posterior probability for the Bayesian analysis.

TABLE 4. The *Trypophloeus* species-groups according to Pfeffer (1995).

<i>T. alni</i> species-group
<i>T. alni</i> (Lindemann, 1875)
<i>T. dejevi</i> Stark, 1936
<i>T. rybinskii</i> rybinskii Reitter, 1895 ¹
<i>T. rybinskii</i> corsicus Eggers, 1912
<i>T. asperatus</i> species-group
<i>T. grandis</i> Schedl, 1964
<i>T. asperatus asperatus</i> (Gyllenhal, 1813)
<i>T. asperatus spiculatus</i> Eggers, 1927
<i>T. asperatus grothi</i> Hagedorn, 1904
<i>T. granulatus</i> species-group
<i>T. klimeschi</i> Eggers, 1915
<i>T. granulatus</i> (Ratzeburg, 1837)
<i>T. tremulae</i> Stark, 1952
<i>T. bispinulus</i> Eggers, 1927
<i>T. palmi</i> Hansen, 1956 ²

Note 1: *T. rybinskii rybinskii* Reitter was published 1895 not 1894 according to Knižek (2011)

Note 2: *T. palmi* was published in 1956 (Hansen 1956)

Species-groups in the genus *Trypophloeus*

Pfeffer (1995) divided the genus *Trypophloeus* into three species-groups based on morphology alone. To the best of our knowledge, this is the first and only published division into species-groups of the genus *Trypophloeus*.

The species of the *T. alni* species-group can be separated from the rest of the *Trypophloeus* species by morphological characters, which often are overlooked. The main characters are the frontal microsculpture and reticulated (a network of fine impressed or elevated transverse lines) upper part of frons and vertex (Figure 4), while the other *Trypophloeus* species has aciculation (minute subparallel scratches closely resembling those made with the point of a needle). Additional features common for the species of the *T. alni* species-group are strongly impressed elytral striae and absence of denticles at the base of elytral declivity. However, some of these features are also shared by some other representatives of the genus *Trypophloeus*.

These morphological characters alone

constitute a weak basis for the division of the genus into two species-groups: *T. alni* species-group (Table 4) and the rest of the species in a *T. binodulus* species-group. This result is also in contrast to our results obtained by the DNA analyses (Figure 1). Based on these DNA analyses we propose that the genus is divided into four species-groups (Table 5, 6, 7, 8). We consider the groups as proposals since we have not carried out morphological studies to support a formal description of the groups. DNA sequences were available for all species in the genus, except *T. grandis* Schedl, 1964. Consequently, we have not proposed any placement of the species in a species-group.

Descriptions and taxonomy

***Trypophloeus borealis* sp. n.** (Figures: 5–9)

Examined material: *Holotype:* FINLAND: Male, Province of Enontekis Lappmark: Kuttanen, 68°26'N/22°43'E, 4.VII.2012, leg/det. Å. Lindelöw. The holotype will be deposited in Swedish Museum of Natural History (Stockholm, Sweden) **SMNH**. *Paratypes:* SWEDEN: 1 ex. Torne Lappmark, Årosjokk, 18.VI.1969, Lars Huggert leg., **SMNH**; 5 exx., Torne Lappmark, Årosjokk. 67°87'N/19°37', 15.VI.2008, leg. et det. Å. Lindelöw, **CÅL**; 26 exx., Torne Lappmark, Årosjokk. 67°87'N/19°37' 5.VI.2009, leg. et det. Å. Lindelöw, **CÅL**; 1 ex. Torne Lappmark, Årosjokk, 67°52' N, 19°22'E, stems of *Salix myrsinifolia*, 15.VI.2008, Å. Lindelöw leg./det., **CÅL**; 3 exx. Torne Lappmark, Akkar. 67°86'N/19°43', 5.VI.2009, leg. et det. Å. Lindelöw, **ZIN**; NORWAY: 8 exx., Region of Troms and Finmark, Karasjok: Jergul, 69°25'N/24°58'E (EIS 166), 3.VII.2012, stems of *Salix myrsinifolia*, leg./det. Å. Lindelöw, **CÅL**; 1 ex., Region of Troms and Finmark, Karasjok, Jergul, RT90 7732/1858. 3.VII.2012. Svartvide. Leg et det. Å. Lindelöw, **CTK**; FINLAND: 8 exx., Province of Enontekis Lappmark: Kuttanen, 68°26'N/22°43'E, 4.VII.2012, stems of *Salix glauca* (Ripvide), 1-2 cm thick, leg. et det. Å. Lindelöw, **CÅL**; 1 ex. Province of Enontekis Lappmark: Kuttanen, 68°26'N/22°43'E,

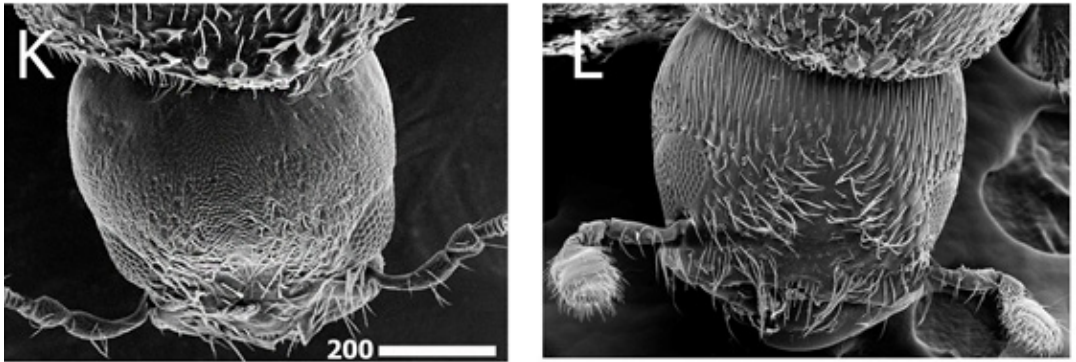


FIGURE 4. The head of *Trypophloeus alni* (Lindemann, 1875) with reticulated frontal surface (K) and *T. tremulae* Stark, 1952 aciculated frontal surface (L).

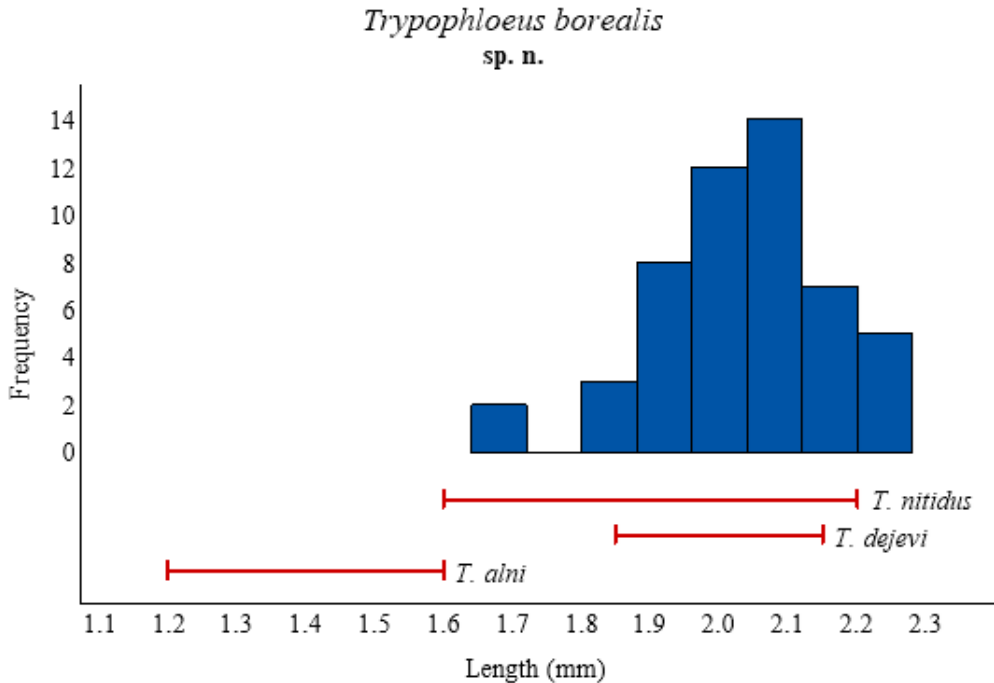


FIGURE 5. The size of 50 specimens of *Trypophloeus borealis* sp. n. were measured and compared to the size of the other species in the *Trypophloeus alni* species group.

4.VII.2012, stems of *Salix glauca* (Ripvide), 1-2 cm thick, leg./det. Å. Lindelöv, CTK.

Description. *Body:* 1.95–2.15 mm long. *Head:* frons convex, with densely and rather shallow, round, partially fusing punctures. Epistoma impressed, surface reticulated and shining; impression is divided into two parts by small median tubercle; epistomal impression have large

fusing punctures. Vertex reticulated, not aciculated. Frontal surface with sparse inclined hair-like yellow setae; setae more abundant at epistomal margin. Eyes oval, with few facets missing behind antennal insertion. Antennae brown, only pedicel yellow. *Pronotum:* 1.1 times wider than long, with rounded posterolateral angles, broadly rounded; 4–8 denticles at anterior margin, arranged in



FIGURE 6. *Trypophloeus borealis* sp. n. Photo Göran Liljeberg.

concentric, transverse rows, highest pronotum point behind middle. Pronotum densely punctured near base and at posterolateral angles, brightly shining, faintly reticulated between punctures and evidently reticulated at flat bottoms of individual points; punctured area is covered by abundant and rather short hairs with their apices oriented forward and reaching the base of adjacent hairs. Anterior tuberculated part of pronotum with hairs directed backwards, with longer hairs at anterior margin of pronotum and at epipleura, surface between crenulations also reticulated but shining. *Scutellum*: flat and shining, strongly punctured, covered with short hairs which are oriented backwards. *Elytra*: more than 1.5 times as long as wide, 1.8 times longer than pronotum, with developed humeral angles; sides subparallel at basal 2/3 and rather narrowly rounded posteriorly. Surface brightly shining, glossy, with very faint reticulation and with strongly developed striae of round punctures, densely set, with less than



FIGURE 7. Habitat for *Trypophloeus borealis* sp. n. in Finland. *Salix glauca* L. is one of the dominating willow plants along the stream.



FIGURE 8. An early stage gallery or maturation feeding of *Trypophloeus borealis* sp. n. in *Salix myrsinifolia*, from the locality in Norway.



FIGURE 9. Exit holes from *Trypophloeus borealis* sp. n. in *Salix glauca*, from Finland.

1/2 of puncture diameter between individual points, interstriae slightly wider than striae, with 3 irregular rows of minute punctures, each puncture bearing short hair-like scale directed backwards; central row of interstitial scales somehow longer, but still less than distance between individual bristles in row. Most of elytra has long, erect and thin hairs. Declivity with inclined hair-like scales, rather gradual, occupying only posterior 1/3 of elytral length, with suture not elevated. Individual inclined scales at elytral declivity are strongly serrate at sides and are pointed at apex, recumbent scales at declivity are also strongly serrate. *Legs*: femur and tibia brown and tarsus are yellowish. A small 8th abdominal tergum is present in males, but not females. This is a common feature of all former Cryphalini, including Trypophloeini.

Diagnosis. Specimens of *T. borealis* **sp. n.** from Northern Fennoscandia differs morphologically only subtly from *T. nitidus* and *T. dejevi*. *T. borealis* **sp. n.** differs from *T. nitidus* by slightly larger size (1.67–2.25 mm vs. 1.7–1.9 mm in *T. nitidus*). The average length of *T. borealis* **sp. n.** was 2,03 mm. In long series of “*nitidus/striatulus*” (at **BEM**) body length was found to be 1.6–2.1 mm). The structure of the frons, which is usually medially impressed in *T. nitidus*, can be very variable.

Etymology. The name borealis is a derivation of boreal, which means “belonging to the north”.

Notes on the biology and development of *Trypophloeus borealis* **sp. n.**

Biology and development in *T. borealis* are very similar to *T. nitidulus* in North America (Furniss 2004, 2013), except different host tree choice. Newly emerged adults perform maturation feeding in cave-like galleries in the bark of living trees/bushes of *Salix*. After hibernation, some adults may leave the host tree and disperse. Suitable trees are colonised for reproduction. In some cases, this may occur in the same gallery in which hibernation took place. Colonisation seems to be related to parts of the tree, which have reduced vitality or damaged or dying tissue. Galleries have been found in the transition zones between dead and alive tissue. Such zones often occur after cracks and other damages caused by snow

during the winter. *T. nitidus* mating is taking place at the bark surface. The female enters the bark first, joined by the male later. The *Trypophloeus* species are monogamous and only one female and one male are found in a gallery. The eggs are laid in a cluster (Furniss 2004) in the gallery. Thus, the female does not make a separate niche for each egg. This behaviour is characteristic in Cryphalini (Wood 1982). The development seems to be irregular. In mid-June parent beetles and larvae were found together in the galleries and in early July, adults, pupae and larvae have been observed (Lindelöw and Kvamme 2013). We assume that the development time can be more than one year due to the short summers and harsh climate in the North. Since galleries can be found in small parts of trees it is likely that the species can develop for several generations in the same trunk when the area of dead tissue increases year by year.

The first Scandinavian record was probably made by sweeping in June in the area where the species was rediscovered in *Salix myrsinifolia* (Lindelöw 2009). In Norway the host tree was also *S. myrsinifolia*. In Finland the species was found in 1–2 cm thick trunks of *Salix glauca* (Lindelöw and Kvamme 2013). *S. myrsinifolia* (= *S. nigricans*) is widely distributed in North Europe, eastwards to the Ob Valley. *S. glauca* is circumpolar and widely distributed, both in Palaearctic as well as Nearctic (Hultén & Fries 1986, Jonsell 2000).

Orange tendrils of fungus protruded from the bark surface have been observed several times (Figure 16) (Lindelöw and Kvamme 2013). They resemble the *Cytospora* sp. that Furniss (2004) observed on the bark surface of *Salix alaxensis* (Andersson), which was colonised by *T. nitidus*. Any possible relation between the fungus and the beetles is unknown. Spores found on the surface of the pronotum of *T. striatulus* did not belong to *Cytospora* sp. (Furniss 2004).



FIGURE 10. *Cytospora* sp. tendrils sprouting from a *Salix myrsinifolia* stem with emerging holes of *Trypophloeus borealis* sp. n. from the Norwegian locality.

***Trypophloeus nitidus* Swaine, 1912 res. n.**

Trypophloeus nitidus Swaine, 1912: 349

=*T. striatulus* auct., nec Mannerheim, 1853: 235 **syn. n.**

=*T. punctipennis* Hopkins, 1915: 37

Examined material. Type material: Holotype: *Trypophloeus striatulus* (Mannerheim, 1853): circle of golden paper // “*Cryphalus striatulus* Mnnhm (=Mannerheim), Kenai J.” farther unreadable in ink handwriting, probably “Russam”, **ZIN**.

Comment. The type of the species has been considered lost, at least not found in Mannerheim collection, Finland (Wood and Bright 1992). However, in 1853 both Alaska and Finland were still parts of Russian Empire and thus deposition of the holotype in main country collection, i.e. in the former capital of the Empire (St. Petersburg) was logical. *Cryphalus striatulus* is the original valid name proposed by Mannerheim for a new species. It was described and really belongs to genus *Cryphalus* and not to genus *Trypophloeus* as many authors thought. It is not a new synonym, but a senior name and *Cryphalus ruficollis* is a new junior synonym of *Cryphalus striatulus*.

Trypophloeus nitidus Swaine, 1912:

Lectotype: ♀, 172 [in red ink on small rectangle]// Weymouth NS [in red ink] **LECTOTYPE** [in black ink, printed] CNC No 9276// *Trypophloeus nitidus* Sw. [handwritten in black ink], female, **CNCI**; Paratype (Paralectotype) 1 ex., Cornell U., No. 292.2// *Trypophloeus nitidus* SW.//on *Alnus* G.E. Sanders

// Weymouth 1912 – n.8, **NHMW**; 1 ex., 2476// [silver rectangular piece of paper]// *Cryphalus nitidus* Swaine **Type series** S.L. Wood (19)56// *Trypophloeus nitidus* Sokanovský det., **ZMMU**.

Trypophloeus punctipennis Hopkins, 1915

Holotype: ♀, //Alta 30.6. Ut.// 7599° [in blue ink] Hopk W. Va [in black ink]// Det. Type [in blue ink] No. Hopk. 3-1-02// Leg. Mounted [on blue paper]// *Trypophloeus punctipennis* Hopk. [in black ink]// *punctipennis* H.3 [in black pencil] *Glyptoderes punctatus* n.sp. [in blue ink] Hopk. [on reverse side]// *Glyptoderes punctipennis* Hopk. [in black ink] Hopk. [printed]// // red rectangle Typ No. 7399, **USNM**.

Additional material. CANADA: 5 exx. 1930 Karl Schedl 737, **NHMW**; 4 exx. 1930 Karl Schedl 759, **NHMW**; Ontario: 4 exx. Biscotasing, *Alnus incana*, 10.V.1930, K.E. Schedl leg/det, **NHMW**; Yukon Territory: 5 exx. Elliot Lake, *Salix*, 25.IX.1987, M.M. Furniss leg., **BEM**; USA, Alaska: 6 exx. Cantwell, *Salix*, 29.VII.1967, M.M. Furniss leg./det., **BEM**; 13 exx. 21 miles E of Cantwell, *Salix*, 30.VIII.1967, M.M. Furniss leg./ S.L. Wood det., **BEM**; 12 exx. Kongakut Riv., Brooks Range, *Salix*, 26.VIII.1974, M.M. Furniss leg./ S.L. Wood det., **BEM**; 3 exx. Shublik Springs. Cunning River, Host *Salix* sp., 8.X.1976, M.M. Furniss leg., **TAMU**; 10 exx. Mount. Mc. Kinley N.P., Igloo camp, *Salix*, 29.V.1977, M.M. Furniss leg./det., **BEM**; 4 exx. Fairbanks, *Salix*, 30.VIII.1977, L.Bordelon leg./ M.M. Furniss det., **BEM**; USA, Utah: 2 exx. S36 T12 NR2E, Logan Canyon., el. 7,000 Ft. *Salix scouleriana*, 17.VII.1946, S.L.Wood leg., **SEM**; 1 ex. Little Bery, Logan Canyon., el. 8,000 Ft., *Salix scouleriana* 17.V.1947, S.L. Wood leg., **SEM**; 1 ex. Logan Canyon, *Salix scouleriana*, 28.V.1949, S.L.Wood, **SEM**; USA, Idaho: 10 exx. Coeur d’Alene *Alnus* sp., 4.XII.1916 Evenden JC Colr., **BEM**; 2 ex. Poison Creek, 20 mi SW Grandview, *Salix* 31.VII.1967, M.M. Furniss leg., D.M. Anderson det., **BEM**; USA, Oregon: 15 exx. Hot Springs C.G. Hart Mtn. Refuge, Lake Co. *Salix scouleriana* 14.VIII.1990 M.M. Furniss, J.B. Johnson leg, M.M. Furniss det., **BEM**

General distribution. North America, including Canada (Newfoundland, Nova Scotia,

Quebec, Yukon), Alaska and mountains of western USA (Colorado, Idaho, Minnesota, Oregon, Utah) (Wood and Bright, 1992).

Comment. We were not able to find the reference indicating the introduction of the species to Hungary (*cf.* Knížek, 2011).

Host trees. *Alnus crispa*, *A. rugosa*, *Salix scouleriana*, *S. alaxensis*.

Re-description. *Body:* length 1.6–2.2 mm, 2.7 (2.4–3.0) times as long as wide, black or dark brown, brightly shining. *Head:* frons convex, with Y-shaped impression from upper level of eyes to antennal insertions; surface finely reticulated from upper level of eyes to vertex, shallowly punctured below; pubescence consisting of inconspicuous sparse hairs. *Pronotum:* widest at base, distinctly transverse, 0.65–0.75 as long as wide; summit slightly behind middle; asperities in front of summit, rather large and abundant, forming concentric rows in anterior half; anterior margin with four to eight contiguous teeth. Surface of lateral and posterior areas shining, punctures rather close, coarse and deep; covered with short hair-like setae, slightly longer ispiratete area. *Elytra:* glossy, 1.6–2.0 times as long as wide; punctures on striae and interstriae deeply impressed in anterior two thirds, becoming smaller and shallow near declivity; interstriae with small granules on declivity; surface covered with recumbent hair-like strial and interstitial setae, becoming more scale-like at the declivity, and with rows of erect elongated scales on interstriae throughout elytral length.

Diagnosis. Distinguished from other species due to reticulated surface of the upper portion of frons and vertex; from *T. alni* and *T. dejevi* by more weak punctures in basal half of pronotum and declivity, by elytral vestiture, and by brightly shining body. *T. nitidus* differs from the North American species *T. populi*, *T. salicis*, and *T. thatcheri* by strong puncturation of elytra organized in rows. These rows of punctures on elytra extending at least two-thirds from the elytral base to declivity. The scale-like pubescence is only present at the declivity.

***Trypophloeus alni* (Lindemann, 1875)**

Cryphalus alni Lindemann, 1875a: 136

= *Trypophloeus holdhausi* Wichmann, 1912: 186

Examined material:

***Trypophloeus alni* (Lindemann, 1875). Type material:** Lindemann's syntypes of *Cryphalus alni* are not preserved in ZMMU where they were expected to be kept. However, some of Lindemann's specimens were located in NHMW. Lectotype is designated here from the NHMW. Lectotype: ♀ *Trypophloeus alni* (Lindemann, 1875). Mosqua//Lindemann//Collect Türk.// *Cryphalus alni* Lind. **Typ** [in handwriting] // **TYPUS** [on red paper, printed] // **Lectotypus** *Cryphalus alni* Lindemann, 1875 designated by Mandelshtam (date marked on label as 2006, but in fact lectotype is designated only in the current paper)//*Trypophloeus alni* (Lindemann, 1875) det. Mandelshtam, 2006 **NHMW**; Paralectotypes. 7 ex. with same collecting label: Lindemann//Mosqua// **NHMW**.

***Trypophloeus holdhausi.* Holotype:** *Trypophloeus holdhausi* Wichmann, 1912: Holdhaus, Campo-Grosso// bei Rovereda, Süd Tirol// **TYPUS** [printed, on red paper] // *Trypophloeus Holdhausi* n. sp. ♀ **Type** 1912 det. Wichmann, 1912//Holdhausi Wichm. Ti.m// *Trypophloeus alni* (Lindemann, 1875) det. Mandelshtam, 2006. Type is located in **NHMW**.

Additional material. ESTONIA: 2 exx. Estland, Leliland, 10.VIII.1937, J. Hristian, 113, **NHMW**. RUSSIA, Leningrad Province: 2 exx. Lesnoy, Petrograd, 25.IV.[1]915, Spessivtsev leg., **ZIN**; 2 exx. Beloostrov, Sestra River Bank, river-flooded forest, forest with *Alnus* and *Prunus padus*, under bark of fallen *Alnus incana* thick branches, 23.IV.1990, M.Yu. Mandelshtam leg., **ZIN**; 11 exx. Orzhitsy, Lomonosov District, park, under bark of fallen stem of *Alnus incana*, 20.VIII.2001, M.Yu. Mandelshtam leg., **ZIN**; 1 ex. RUSSIA, Pskov Province, Sebezh District, Sebezh National Park, Osyno Forestry, under bark of *Alnus incana* cut stem, VII.2002, Mandelshtam leg., **ZIN**; RUSSIA, Arkhangelsk Province: 12 exx. 1851 alder, Vel'sk, Vologda Government, 23.VIII.1929, G.K. Pjatnitskiy leg., **ZIN**; 33 exx. under number 1855, 3.IX.1929; 57 exx. under number 1854, **ZIN**; RUSSIA, Yaroslavl Province, 3 exx. Poshekhonsk

forestry, Yaroslavl Government, standing dead willow, 20 years, 17.VII.1928, G. Pjatnitskiy leg., **ZIN**; RUSSIA, Moscow Province: 1 ex. Bol'shevo Station, in the bark of stem of alder, 15.IV.1925, S. Shorokhov leg., **ZIN**; 1 ex. **ZMMU**; 1 ex. Bol'shevo Station, in the bark of growing drying alder, 06.VI.1925, S. Shorokhov leg., **ZIN**; 2 exx. Bol'shevo Station, in the bark of growing drying alder, 14.VI.1925, S. Shorokhov leg., **ZMMU**; 2 exx. Bol'shevo Station, alder, 14.VI.1926, **ZIN**; 2 exx. Zvenigorod Biological Station of Moscow State University, window trap, 23.V.1981, M. Samkov leg. (V. Gratchev coll.), **ZMMU**; 1 ex. Zvenigorod Biological Station of Moscow State University, 30.VI.1981, M. Samkov leg. (V. Gratchev coll.), **ZMMU**; 1 ex. Zagorsk District, Malinniki village, waterfall "Gremyachiy", aspen stem, 22.V.1982, A. Petrov leg., **ZMMU**; 1 ex. Zvenigorod, 22.III.1989, M. Yu. Mandelshtam leg., **ZIN**; 3 exx. Morozky, window trap on the elm No. 2, 25.IV–18.V.1994, Nikitsky leg., **ZMMU**; 2 exx. Mozhaiskiy District, near village Osiritsi, window trap 5, 22.IV.–28.V.2009 Nikitsky leg., **ZMMU**; RUSSIA, Bryansk Province: 4 exx. Bryansk, Orlov Gouvernement, 22.VII.[1]926, V. Stark leg., **ZIN**; RUSSIA, Permsk Province: 1 ex. Middle Ural, environment. of Gubakha, Molotov Province (nowadays Permsk Province), alder, water-flooded forest of Kos'va River, square. 265, 28.XI.1952, Zinovjev leg., **ZIN**; 2 exx. environment. of Molotov (nowadays – Perm), Mysy village, 07.V.1953, G. Zinovjev leg., **ZIN**; 9 exx. environment. of Molotov (nowadays – Perm), Mysy village, 13.V.1953, G. Zinovjev leg., **ZIN**; 3 exx. Mysy village, Krasnok[utskiy] District, alder, 07.VI.1953, G. Zinovjev leg., **ZIN**; RUSSIA, Kemerovo Province: 1 ex. Kemerovo, on willow, "intermediate form between *T. alni* and *T. rybinski*" [B. Sokanovskij note], **ZMMU**; 3 exx. Aspen Plateau, willow, M. Lurie leg.; "*Tr. alni* v. *rybinski* Rtt. B. Sokanovskij det. 57", **ZIN**; PATRIA IGNOTA: 2 exx. 1930 Karl Schedl 135, **NHMW**; 8 exx. 1930 Karl Schedl 741, **NHMW**.

General distribution: Austria, Belarus, Estonia, Finland, Germany, Norway, Poland, Switzerland, European part of Russia (Northern and Central Territories), West Siberia (Knížek 2011, Kvamme & Lindelöw 2014).

Comments. Reports of the species from Italy is erroneous. It is based on incorrect assignment of Campo-Grosso to Italy, but in fact this locality is in Switzerland.

T. alni is listed from China (Hua 2002, Knížek 2011), including Xinjiang and Sichuan. The general distribution indicates that this most probably is erroneous and might refer either to *T. dejevi* or to a still undescribed species (cf. Knížek 2011).

No specimens from Ukraine were found in available collections.

Host trees: *Alnus glutinosa*, *A. incana*. Much more common in *A. incana*.

In China *T. alni* is mentioned to infest *Alnus* and *Populus davidiana* (Hua 2002). However, since we evaluate the occurrence of *T. alni* in China to be doubtful, we do not delete this information until new documentation is available.

Re-description. *Body:* small, slim and strongly elongated, 1.2–1.6 mm, 3.0–3.25 times as long as wide, dark brown to black. *Frons:* weakly convex, shallowly reticulated from upper level of eyes to vertex; vertex entirely reticulated. Frontal pubescence consists of inconspicuous sparse hairs, vertex glabrous. *Pronotum:* triangular of equal length and width at base, widest posterior of tuberculated area, summit at middle. Asperities in front of summit abundant, rather small, conical, their height is equal to width at base, apically sharpened, basically not fused, not arranged in regular rows. Number of tubercles at pronotal, anterior margin variable. Pronotal vestiture consists of short, fine hairs with hair-like scales intermixed, the latter more abundant nearby base. Setae directed backward at tuberculated area and forward at the posterior area. *Elytra:* as long as wide, 2.2 (2.08–2.33), just a little wider than pronotum (1.0–1.1), sides parallel on basal two thirds, narrowly rounded at apex. Striae distinctly impressed throughout their length, with elongated punctures; interstriae slightly raised. Strial punctures with short hair-like setae, interstriae with ground vestiture of recumbent hair-like setae replaced with scale-like setae at declivity and each with a single row of long erect hair-like scales.

Diagnosis. Rather similar to *Trypophloeus dejevi* and *T. nitidus* and sharing with these two

species the reticulated (not aciculated) surface of the upper portion of frons and vertex. Can be distinguished from both species by more elongated elytra and by acute, not merging asperities at tuberculated area. *T. rybinskii*, one more species without spines and granules at male elytral declivity, has aciculated surface of the upper portion of frons, indistinct elytral striae and much stouter body. The smallest of the species in the species group (Figure 5).

Trypophloeus dejevi Stark, 1936

T. dejevi Stark, 1936: 152

T. niger Stark, 1936: 152 **nov. n.**

T. dejevi Eggers, 1942: 31

Type material: Lectotype: “Sajan Mts., Margasan River, 22.VIII.31, S. Deev leg.”// “Mixed forest, willow, stem, 56”// “*Trypophloeus dejevi* Stark, **types**, d[et]. Stark, 1933”// “**Lectotypus** *Trypophloeus dejevi* Stark, det. Michalski 1965”, **ZIN**; paralectotypes: 7 exx. with the same geographic label, one bearing an additional label “*Trypophloeus deevi* Stark, **nov. types**”; 2 exx. with labels “Sajan Mts., Barun-Ingasun River (in Michalsky 1969, name of river is given erroneously as Barun-Intarun), 14.IX.31., S. Deev leg.”, “Mixed forest, alder, stem 79”// “Paralectotypus”, **ZIN**.

Comment. Michalski designating the lectotype and used the latinized version of the term on the label, and his label is cited here in full. In addition to the types labeled by J. Michalski there are more specimens in V.N. Stark collection preserved at **ZIN**. Eight of these specimens have the same label as the **lectotype**, and 7 specimens carry the labels: “Sajan Mts., Margasan River 24.VIII.31, S. Deev leg.”, “Mixed forest, willow, stem 62”.

Trypophloeus niger Stark, 1936. **Type material:** Lectotype: “Vladivostok Reg., Maikhe forestry, 2.VIII.1930”, “159”, “species? from willow”, “*Trypophloeus niger* Stark, types d. Stark, 1933” “**Lectotypus** *Trypophloeus niger* Stark, det. J. Michalski, 1965”, **ZIN**; paralectotype: “Vladivostok Reg., Maikhe forestry, 2.VIII.1930”, “159”, “species? V. Stark det. 1931”.; “159, bank of Maikhe River on willow *Salix* 2.VIII.1930, Lyubarskyi”, **ZIN**.

Comment. The original description was based on only two **syntypes**. No more specimens of this species are known so far.

Additional material: RUSSIA, Irkutsk Province: 1 ex. Sajan Mountains, Margasan River, mixed forest, willow, stem 56, 22.VIII.1931, S. Deev leg., **IBSS**; 1 ex, Sajan Mountains, Barun-Ingasun River, mixed forest, alder, stem 79, 14.IX [19]31, **IBSS**; 1 ex. Baical (sic!), Sajan, 14.IX.1931 *Salix*, Dejev leg., **NHMW**; 1 ex. Baikal, *Salix*, 16.IX.1931, Cotype, Dejev leg., **NHMW**; 7 exx. Nizhneangarsk, alder, without date and collector name, **ZMMU**; RUSSIA, Chita Province: 4 exx., Kodar Mts., near Sul’han River, 1600–1900 m., 10–17.VII.1997, A. Petrov leg., **ZIN**; RUSSIA, Kamchatka Province: 5 exx. 28.VII.1958, Kurentsov et Kononov leg., **IBSS**; 2 exx. 13.VIII.1958, Kurentsov leg., **IBSS**; 2 exx. 14.VIII.1958, Kurentsov leg., **IBSS**; 3 exx. 17.VIII.1958, Kurentsov et Kononov leg., **IBSS**; 4 exx. 27.VIII.1959, D. Kononov leg., **IBSS**; 8 exx. 6.VII.1960, Ivliev leg., **IBSS**; 7 exx. 8.VII.1960, Kupyanskaya leg., **IBSS**; 12 exx. 9.VII.1960, Lisichenko leg., **IBSS**; 6 exx. 11.VII.1960, Ivliev leg., **IBSS**; 18 exx. 15.VII.1960, Kupyanskaya leg., **IBSS**; 2 exx. 17.VII.1960, Kupyanskaya leg., **IBSS**; 7 exx. 18.VII.1960, Kupyanskaya leg., **IBSS**; 2 exx. without collector name, **IBSS**; 9 exx. 26.VII.1960, Lisichenko leg., **IBSS**; 1 ex. 8.VIII.1960, Lisichenko leg., **IBSS**; 14 exx. 8.VIII.1960, Kupyanskaya leg., **IBSS**; 5 exx. 10.IX.1960, Lisichenko leg., **IBSS**; additional 69 exx. without precise locality, collected in VII–IX 1958–1960 by different collectors, **IBSS**; RUSSIA, Sakhalin Province: 5 exx. Sakhalin, 06.VIII. [19]51, G. Krivolutskaya leg., **ZMMU**; 53 exx. Ulegorsk District, environs of Kotan settlement, sea shore, from alder, 6.VIII.1951, Krivolutskaya leg. (erroneously labeled as syntypes of *Eocryphalus zachvatkini* Krivolutzkaja, collected at same place and date), **ISEA**; 13 exx. Sakhalin, alder, without date, G. Krivolutskaya leg., **ZMMU**; RUSSIA, Amur Province, 1 ex. Zeisky Nature Reserve, kordon 52-nd km. 25.VII.1978, leg. Zimenko (N. B. Nikitsky coll.), **ZMMU**; RUSSIA, Primorsk Territory: 3 exx. Lesozavodsk town, from aspen, Naberezhnaya street, 24.VIII. [19]66, A.N. Kypyanskaya leg., **IBSS**; 5 exx.

Vladivostok, on *Populus tremula*, 16.IX.2000, M. Mandelstam leg., ZIN.

General distribution: Russia: Eastern Siberia (Sajan Mts., Cisbaikalia, Transbaikalia), Sakhalin, Kamchatka, Amur Province, Primorsk Territory, Mongolia (Ulan-Bator, 8.VII.1956, on willow, leg. V.P. Gretchkin, cf. Sokanovsky 1960), (Knížek 2011).

Host trees: *Salix sachalinensis*, *Salix* spp., *Alnus* sp., and *Populus tremula*.

Re-description. *Body:* length – 1.85–2.15 mm, 2.7 (2.6–2.9) as long as wide. Body black, faintly shining. *Head:* antennae brown, frons slightly impressed from the middle to epistoma; surface finely punctured, more densely at lower half; reticulated from upper level of eyes to vertex; vertex entirely reticulated. *Pronotum:* widest near base, summit slightly behind middle, distinctly elevated; asperities coarse and forming concentric rows in anterior half; anterior margin with six teeth; surface rough, less densely punctured at posterior half, covered with mixture of hair-like setae and scarce elongated scales. *Elytra:* 1.85–2.0 times as long as wide, striae punctures distinctly impressed throughout elytral length; interstriae with shallow punctures; covered with recumbent hair-like setae replaced with scale-like setae on declivity; 2nd and 3rd interstriae on declivity slightly raised. *Legs:* legs brown colored.

Diagnosis. *T. dejevi* can be distinguished from closely related *T. alni* and *T. nitidus* by stouter body, coarse asperities in anterior half of pronotum, distinct, deep punctures on elytra and pronotum, and by elytral vestiture.

Discussion

The results confirm the difficulties using classical taxonomy and morphological characters alone as a basis for description and identification of *Trypophloeus* species. Many of the morphological characters are not easy to see. It is necessary to have a series of specimens to make correct identifications, if possible, by morphological characters alone. In addition, the morphological characters show variations and overlap between the species. Thus, we support our conclusions on

a combination of classical taxonomy and analyses based on DNA sequences. Morphological characters in the genus *Trypophloeus* should only be used with precaution. The characters described should be used as a guideline more than an absolute answer. There are still many details and questions in the genus *Trypophloeus* that need to be studied.

The historical confusion can easily be illustrated by the inconsistent use of names. The nomenclatorial changes have been done in agreement with the code (ICZN 1999).

The following nomenclatorial changes are presented for the closest related species of *T. borealis* **sp. n.** Mannerheim's type of *Cryphalus striatulus* was previously considered lost but was rediscovered in the collections of the Zoological Institute in St. Petersburg (ZIN). Examination of the type showed that it belongs to the genus *Cryphalus* and not to the genus *Trypophloeus* (cf. Wood & Bright 1992). Consequently, *Trypophloeus striatulus* (auct. nec Mannerheim, 1853) must be transferred to the genus *Cryphalus* and called *Cryphalus striatulus* Mannerheim, 1853 **res. n.** *Cryphalus striatulus* is a senior synonym over *Cryphalus ruficollis* Hopkins, 1915 **syn. n.** The combination *Trypophloeus striatulus* is thus not a valid name. *Trypophloeus nitidus* Swaine, 1912 **res. n.** is resurrected as a valid name for this North-American species (cf. ICZN 1999). This is the reason why we use *T. nitidus* for this American species from Alaska recognized by most authors as *T. striatulus*.

Examination of the two syntypes of *T. niger* Stark, 1936 at Zoological Institute, St. Petersburg, show that this is a synonym of *T. dejevi* Stark, 1936. *Trypophloeus niger* is described in the same paper, but after *T. dejevi*, so it becomes a junior subjective synonym. *Trypophloeus dejevi* Stark 1936 has therefore priority over *Trypophloeus niger* Stark, 1936 **syn. n.**

We are aware that the names *T. asperatus*, *T. granulatus*, *T. granulatus grothi* and *T. binodulus* following the sequences generated from open sources are all linked with nomenclatorial problems. We use these names unchanged as used originally with the sequences. On figure 1 the name *T. asperatus* follows sequences in the

outgroup and one sequence grouping together with *T. granulatus*/*T. granulatus grothi*. These are misnamings. *Trypophloeus* species cannot both be a member of the genus and an outgroup at the same time. These specimens are *Cryphalus asperatus*. The name *T. asperatus* on figure 2 is representing *T. binodulus*. *T. binodulus* (Ratzeburg, 1837) is the the valid name for *Cryphalus asperatus*. However, we leave the rest of these questions open since they are outside the scope of the presented paper.

Apart from Pfeffer (1995), little attention has been paid to species groups in the genus *Trypophloeus*. We believe that all the other unsolved taxonomic and nomenclatorial questions have overshadowed this question, also because species-group is not regulated by the code (ICZN 1999). The proposal of four species-groups is mainly based on the results of the DNA analyses due to small morphological differences. The result is a proposal since we did not carry out a study of differences in morphology between the species-groups. Neither was the question of subgenera studied. However, our results based on the DNA sequences provided strong support of the *T. alni* species-group as a monophyletic group.

The finding of a *Trypophloeus* species in *Salix myrsinifolia* and *S. glauca* in Northern Fennoscandia (Lindelöw 2009, Lindelöw & Kvamme 2013) raised the question about species identity. However, the distance between the Scandinavian Peninsula and the nearest known locality of *T. dejevi* from Saján Mountains, Russia, is enormous. It was first assumed that this was a transpalaeartic species being overlooked in Northern Europe and Siberia. The biology of the Nearctic *T. nitidus* as described by Furniss (2004), combined with the morphological similarity, made it necessary to test the relationship between these species. Our phylogenetic analyses found two monophyletic clades with high support. The first clade contained *T. alni* and *T. dejevi*, and the second clade contained *T. nitidus* and the North-Scandinavian specimens. Our analyses based on ML and Bayesian suggest that the specimens of *T. borealis* **sp. n.** form a monophyletic lineage with high bootstrap and Bayesian support. The pairwise nucleotide difference between *T. dejevi* and *T. borealis* **sp. n.** was on average 9.76%, well above

the range of 2% estimates suggested in previous studies of other insects, including Coleoptera (Cognato 2006, Cognato & Sun 2007, Knee *et al.* 2012) and other estimates of cryptic species in Lepidoptera (Hebert *et al.* 2004). In addition, our estimates of intraspecific pairwise nucleotide difference for those species where we have multiple specimens did not overlap with intraspecific differences, and it fall below estimates from the literature in other insect groups (Cognato 2006). The level of intraspecific variation depends on the group and even certain species. Generally, the border value within COI at least in insects begins from 2% (Cognato 2006) and depending on the additional evidence can be used for both intra- and interspecific levels. The low difference within the nuclear ribosomal gene 28S rDNA is no surprise as far as nuclear genes show better resolution at higher phylogenetic levels and usually insufficient at the species level. However, even within 28S rDNA gene, we obtained some differences that may indicate a certain divergence of the species as well. Thereby, we believe that the combination of all characters (molecular, morphological and biological) is sufficient for the description of the new species.

All these results support our morphological distinction and grouping of the *T. alni* species-group and provide support for the recognition of *T. borealis* **sp. n.** as a new species within *Trypophloeus*. We therefore conclude that this North-Scandinavian *Trypophloeus* species is undescribed. Both the morphology and the biology of the two closely related species indicate a common ancestry. Our molecular analyses suggest a close relationship between *T. borealis* **sp. n.** and the North-American species *T. nitidus*.

Jordal and Knížek (2007) demonstrated that the North European *Crypturgus subcribrosus* Eggers, 1933 is more closely related to Nearctic *Crypturgus* species than to any European species. This demonstrate an ancient faunal relationship between the fauna of Palaeartic and Nearctic. This pattern seems to be the same between *T. nitidus* and *T. borealis* **sp. n.** According to Wood (1982, page 850) the genera *Cryphalus*, *Trypophloeus* and other genera in the tribe Cryphalini have Eurasian origin and reached North America recently. We

have not found Wood's basis for this conclusion since he does not mentioned references. Thus, we cannot support or dispute, but leave the question open. So far, *T. borealis* sp. n. has only been found in northern Fennoscandia. However, we expect the species to be distributed in Russia.

T. borealis sp. n. is not a strictly monophagous species. The known host tree species indicates that *T. borealis* sp. n. may have a much wider distribution than known today and that also other closely related *Salix* species can be utilized as host trees.

Acknowledgements. A special thank to Malcolm Furniss (Moscow, Idaho, USA) for help to get specimens, constructive input and encouragements. Dr. Nikolay B. Nikitsky (ZMMU, Moscow) and Dr. Alexander Petrov (Institute of Forestry, Russian Academy of Sciences, Moscow) for permit to study *Trypophloeus* specimens in their collections. Dr. Vladimir Gusarov (University Museum of Oslo, Norway) and Dr. Natalia Vandenberg kindly assisted in getting specimens, including types, from Snow Entomological Museum in Lawrence (Kansas) and USNM (Washington) respectively. Dr. Bjarte Jordal (University Museum of Bergen, Norway) has kindly supplied some of COI sequences for Russian *Trypophloeus* specimens. Dr. Harald Schillhammer and the late Dr. Heinrich Schoenmann provided facilities to study Scolytines in Schedl's collection (Vienna, Austria). Dr. Hugh Cross (University of Otago, Dunedin, New Zealand) and Inger Heldal (NIBIO, Ås, Norway) carried out some of the DNA laboratory and analyzing work. Göran Liljeberg kindly provided the color photo of the *Trypophloeus borealis* imago. Dr. Ferenc Lakatos (Budapest, Hungary) gave us good, constructive comments. M.Yu. Mandelstam's research was supported by Russian Fund for Basic Research grant No. 17-04-00360.

A great thank to all of you!

References:

Borowiec, M. L. 2016. AMAS: a fast tool for alignment manipulation and computing of summary statistics. *PeerJ* 4:e 1660. DOI 10.7717/peerj.1660

Cognato, A. I. 2006. Standard percent DNA sequence difference for insects does not predict species boundaries. *Journal of Economic Entomology* 99, 1037–1045. Review.

Cognato, A. I. & Sun, J. H. 2007. DNA based cladograms augment the discovery of a new *Ips* species from China (Coleoptera: Curculionidae: Scolytinae). *Cladistics* 23, 539–551.

Eggers, H. 1912. Beiträge zur Kenntniss der Borkenkäfer III. *Entomologische Blätter* 8, 113–117.

Eggers, H. 1915. *Trypophloeus klimeschi* nov. spec. *Entomologische Blätter* 11, 188.

Eggers, H. 1927. Seltene und neue palaearktische Borkenkäfer VI. *Entomologische Blätter* 23, 120–123.

Eggers, H. 1942. Zur palaearktischen Borkenkäferfauna. (Coleoptera: Ipidae). VIII. Borkenkäfer aus dem asiatischen Russland. *Arbeiten über Morphologische und Taxonomische Entomologie* 9, 27–36.

Fairmaire, L. 1864. Famille des Scolytides. In: Jacquelin du Val, P.N.C. & Fairmaire, L. *Genera des Coléoptères d'Europe*. Vol. 4. Deyrolle, Paris, pp. 97–112.

Furniss, M. M. 2004. Biology of *Trypophloeus striatulus* (Coleoptera: Scolytidae) in feltleaf willow in interior Alaska. *Environmental Entomology* 33, 21–27.

Furniss, M.M. 2013. Northernmost occurrence of bark beetles and their hosts in the Nearctic. *American Entomologist*. 59, 144–149.

Gyllenhal, L. 1813. *Insecta Svecica descripta a Leonardo Gyllenhal. Classic I. Coleoptera sive Eleuterata. To mil. Pars III*. Scaris: F. J. Leverentz, [4] + 730 + [2] pp.

Hagedorn, J. M. 1904. Revision unserer Pappelborkenkäfer. *Münchener Koleopterologische Zeitschrift* 2, 228–233.

Hansen, V. 1956. Notes on some species of *Hylastes* Er. and *Trypophloeus* Fairm. (Coleopt. Scolytidae). *Entomologiske Meddelelser* 27, 169–185.

Hebert, P.D., Penton, E.H., Burns, J.M., Janzen, D.H. & Hallwachs, W. 2004. Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly *Astraptes fulgerator* Proc Natl Acad Sci U S A. 12, 2004, 101(41):14812-7. Epub 2004 Oct 1.

Hopkins, A. D. 1915. *Classification of the Cryphalinae, with descriptions of new genera and species. United States Department of Agriculture, Report No. 99*. Washington: Government Printing Office, 75 pp. + 4 plates.

Hua, Li-Zhong. 2002. *List of Chinese Insects. Vol. II. Zhongshan (Sun Yat – sen) University Press. Guangzhou*. 10 + 612 pp.

Huelsenbeck, J.P. & Ronquist, F. 2001. MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics*. 17, 754–755.

Hultén, E. & Fries, M. 1986. *Atlas of North European*

- Vascular Plants North of the Tropic of Cancer. I. Introduction. Taxonomic Index to the Maps 1–996.* Koeltz Scientific Books. Kőningstein, Germany. I–XVI, 498 pp.
- ICZN, International Commission on Zoological Nomenclature. 1999. *International Code of Zoological Nomenclature. Fourth Edition adopted by the International Union of biological Sciences.* The international Trust for Zoological Nomenclature, London. ISBN: 0 85301 006 4. Xxix + 306 pp.
- Johnson, A. J., McKenna, D. D., Jordal, B. H., Cognato, A. I., Smith, S. M., Lemmon, A. R. & Hulcr, J. 2018. Phylogenomics clarifies repeated evolutionary origins of inbreeding and fungus farming in bark beetles (Curculionidae, Scolytinae). *Molecular Phylogenetics and Evolution* 127, 229–238.
- Johnson, A. J., Hulcr, J., Knížek, M., Atkinson, H., T. H., Mandelshtam, M. Y., Smith, S. M., Cognato, A. I., Park, S., Li, Y. & Jordal, J. B. 2020. Revision of the Bark Beetle Genera Within the Former Cryphalini (Curculionidae: Scolytinae). *Insect Systematics and Diversity* 4(3): 1; 1-81. DOI: 10.1093/isd/ixa a002
- Jonsell, B. (Ed.) 2000. *Flora Nordica. Volume 1. Lycopodiaceae to Polygonaceae.* The Bergius Foundation and Royal Swedish Academy of Sciences. Stockholm. 344 pp. ISBN 917190 033 0.
- Jordal, B.H. & Knížek, M. 2007. Resurrection of *Crypturgus subcibrosus* Eggers 1933 stat. n., and its close phylogenetic relationship to Nearctic *Crypturgus* (Coleoptera, Scolytinae) *Zootaxa* 2007. Vol. 606: 41–50
- Jordal, B.H., Sequeira, A.S., & Cognato, A.I. 2011. The age and phylogeny of wood boring weevils and the origin of subsociality. *Molecular Phylogenetics and Evolution* 59, 708–724.
- Jordal, J.B. & Cognato, A.I. 2012. Molecular phylogeny of bark and ambrosia beetles reveals multiple origins of fungus farming during periods of global warming. *BMC Evolutionary Biology* 12, 133. <http://www.biomedcentral.com/1471-2148/12/133>
- Jordal, J.B. & Kambestad, M. 2014. DNA barcoding of bark and ambrosia beetles reveals excessive NUMTs and consistent east-west divergence across Palaearctic forests. *Molecular Ecology Resources* 14, 7–17. doi:10.1111/1755-0998.12150
- Knee, W., Beaulieu, F., Skevington, J.H., Kelso, S., Cognato, A.I. & Forbes, M.R. 2012. Species boundaries and host range of tortoise mites (Uropodoidea) phoretic on bark beetles (Scolytinae), using morphometric and molecular markers. *PLoS ONE* 10, 1–15.
- Knížek, M. 2011. Scolytinae. pp. 204–251. In: Löbl, I. & Smetana, A. (Eds.), *Catalogue of Palaearctic Coleoptera, Vol. 7.* Apollo Books, Stenstrup, 373 pp. <http://dx.doi.org/10.1603/008.104.0301>
- Kvamme, T. & Lindelöw, Å. 2014. Correction and comments to the Norwegian part of the Palaearctic list of bark beetles (Coleoptera, Curculionidae, Scolytinae). *Norwegian Journal of Entomology* 61, 201–212.
- Larsson, A. 2014. AliView: A fast and lightweight alignment viewer and editor for large datasets. *Bioinformatics* 30, 3276–3278.
- Lindelöw, Å. 2009. *Trypophloeus dejevi* (Stark 1936) – ny barkborre (Coleoptera, Scolytinae) i Europa. [*Trypophloeus dejevi* (Stark 1936) – a new bark beetle (Coleoptera, Scolytinae) in Europe]. *Entomologisk Tidskrift* 130, 81–84.
- Lindelöw, Å. & Kvamme, T. 2013. *Trypophloeus dejevi* (Stark, 1936) (Coleoptera, Curculionidae) – a new bark beetle species in Norway and Finland. *Norwegian Journal of Entomology* 60, 90–94.
- Lindemann, K. 1875. Beiträge zur Kenntniss der Borkenkäfer Russlands. *Bulletin de la Société Impériale des Naturalistes de Moscou* 49, 131–146.
- Mannerheim, C. G. 1853. Dritter Nachtrag zur Käfer-Fauna der nord-amerikanischen Laender des Russischen Reiches. *Bulletin de la Société Impériale des Naturalistes de Moscou* 26, 95–273.
- Marchetti, S.B., Worrall, J.J. & Eager, T. 2011. Secondary insects and diseases contribute to sudden aspen decline in southwestern Colorado, USA. *Canadian Journal of Forest Research*, 41, 2315–2325, <http://www.nrcresearchpress.com/doi/full/10.1139/x11-106>
- Nguyen, L.T., Schmidt, H.A., Von Haeseler, A. & Minh, B.Q. 2015. IQ-TREE: A fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Molecular Biology and Evolution* 32, 268–274. doi: 10.1093/molbev/msu300
- Palm, T. 1950. Anteckningar om svenska skalbaggar. V. *Entomologisk Tidskrift* 71, 129–143.
- Pentinsaari, M., Herbert, P.D.N. & Mutanen, M. 2014. Barcoding beetles: A regional survey of 1872 species reveals high identification success and unusually deep interspecific divergences. *PLoS One* 9(9). doi: 10.1371/journal.pone.0108651.
- Pfeffer, A. 1995. *Zentral- und westpaläarktische Borken- und Kernkäfer. Coleoptera: Scolytidae, Platypodidae.* Pro Entomologia, c/o Naturhistorisches Museum Basel. 310 pp. ISBN 3-9520840-6-9.

- Rambaut, A. 2016. FigTree ver. 1.4.3. Oxford: Department of Zoology, University of Oxford.
- Rambaut, A., Drummond, A.J., Xie, D., Baele, G. & Suchard, M.A. 2018. Posterior summarisation in Bayesian phylogenetics using Tracer 1.7. *Systematic Biology* 0, 1–5.
- Reitter, E. 1895. Bestimmungstabelle der Borkenkäfer (Scolytidae) aus Europa und den angrenzenden Ländern. 31 Heft. *Verhandlungen des Naturforschenden Vereines in Brünn* 33, 36–97.
- Ratnasingham, S. & Hebert, P.D.N. 2007. BOLD: The Barcode of Life Data System (www.barcodinglife.org). *Molecular Ecology Notes* 7, 355–364.
- Ratzeburg, J.T.C. 1837. *Die Forst-insecten oder Abbildung und Beschreibung der in den Wäldern Preussens und der Nachbarstaaten als schädling oder nützlich bekannt gewordenen Insecten. Erster Theil. Die Käfer*. Berlin Nicolai, x + 4 + 202 pp. + 21 plates.
- Resch, M.C., Shrubovych, J., Bartel, D., Szucsich, N.U., Timelthaler, G., Bu, Y. & Pass, G. 2014. Where taxonomy based on subtle morphological differences is perfectly mirrored by huge genetic distances: DNA barcoding in protura (hexapoda). *PLoS ONE*, 9, e90653.
- Ronquist, F. & Huelsenbeck, J.P. 2003. MrBayes 3: Bayesian Phylogenetic Inference under Mixed Models. *Bioinformatics*. 19, 1572–1574. <https://doi.org/10.1093/bioinformatics/btg180>
- Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D.L., Darling, A., Höhna, S. & Huelsenbeck, J.P. 2012. MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61, 539–542.
- Schedl, K.E. 1964. Borkenkäfer des nordwestlichen Afrika. 227. Beitrag zur Morphologie und Systematik der Scolytoidea. *Notulae Entomologicae* 44, 94–100.
- Sikes, D.S., Bowser, M., Morton, J.M., Bickford, C., Meierotto, S. & Hildebrandt, K. 2017. Building a DNA barcode library of Alaska’s non-marine arthropods. *Genome* 60(3), 248–259. Doi: 10.1139/gen-2015-0203
- Silfverberg, H. 2010: Enumeratio renovata Coleopterorum Fennoscandiae, Daniae et Baltiae. *Sahlbergia* 16, 1-144. ISSN 1237-3273.
- Sokanovsky B.V. 1960. On the taxonomy and distribution of bark beetles (Coleoptera, Ipsidae) in the USSR and adjacent countries. II. *Contribution to bark beetle fauna of Mongolian People’s Republic*. // *Revue d’Entomologie de l’URSS*, XXXIX, 3, 674–678 (in Russian, with English summary, p. 678).
- Stark, V.N. 1936. Novye vidy koroedov iz Aziatskoi chasti SSSR [Neue Borkenkäferarten aus dem asiatischen Teile der USSR]. *Bulletin of the Far Eastern Branch of the Academy of Science of the USSR* 18, 141–154.
- Stark, V.N. 1952. *Koroedy* [Bark Beetles]. *Fauna SSSR, Novaya Seriya, No. 49, Zhestkokrylye. Tom. 31*. Moskva, Leningrad: Zoologicheskii Institut Akademii Nauk SSSR. 463 pp. (In Russian).
- Swaine, J.M. 1912. New species of the family Ipsidae (Coleoptera). *Canadian Entomologist* 44, 349–354.
- Tamura, K., Stecher, G., Peterson, D. & Kumar, S. 2013. MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. *Molecular Biology and Evolution* 30, 2725–2729.
- Vega, V.J., Mariño, Y.A., Deynes, D., Greco, E.B., Bright, D.E. & Bayman, P. 2020. A beetle in a haystack: Are There Alternate Hosts of the Coffee Berry Borer (*Hyphenemus hampei*) in Puerto Rico? *Agronomy* 10(2). doi: 10.3390/agronomy10020228.
- Wichmann, H.E. 1912. Beschreibung eines neuen *Trypophloeus*. *Wiener Entomologische Zeitung* 31, 186.
- Wood, S.L. 1954. A revision of North American Cryphalini (Scolytidae: Coleoptera). *The University of Kansas Science Bulletin* 36, 959–1089.
- Wood, S.L. 1982. The Bark and Ambrosia Beetles of North and Central America (Coleoptera: Scolytidae), a Taxonomic Monograph. *Great Basin Naturalist Memoirs* 6, 1–1359.
- Wood, S.L. & Bright, D.E. 1992. A Catalog of Scolytidae and Platypodidae (Coleoptera), Part 2: Taxonomic Index Volume B. *Great Basin Naturalist Memoirs* 13, I–II + 834–1553.

Received: 16 November 2020

Accepted: 11 March 2021