

# Expansion of the secondary range of *Polygraphus proximus* Blandford (Coleoptera; Curculionidae, Scolytinae): invasion of Khamar-Daban mountains (Republic of Buryatia)

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The four-eyed fir bark beetle *Polygraphus proximus* Blandford, 1894 (Coleoptera; Curculionidae, Scolytinae) is an alien Far-Eastern stem pest rapidly spreading in the dark coniferous forests of Siberia. In 2021 we conducted a forest pathology research of Khamar-Daban fir forests in south coast of Baikal Lake. The main aim was to inspect the territory for populations of this species. We found three localities with *P. proximus* on two out of four transects and based upon photo materials represented by stuff of the Baikal Nature Reserve revealed another locality inhabited by the species. The characteristics of *P. proximus* populations such as occurrence, production, population density and the population growth rate in new localities were estimated. Since of our previous forest pathological inspection in 2014 it had spread eastward in Baikal region by at least 95 km. The highest site of *P. proximus* population was the slope of the valley of the Bolshoi Mamai River at an altitude of 1030 m above sea level. The estimated average speed of spread was about of 13-14 km per year. However, the most presumable invasion pathway of this species is the Trans-Siberian Railway. Thus, genetic analysis of sampled insects for the COI marker (mtDNA) revealed haplogroup I that previously was found as dominant variant in populations of Kemerovo and Tomsk Provinces. Despite the scattered distribution of *P. proximus* in Khamar-Daban fir forests the high population growth rate allows us to expect the formation of outbreaks during next decade.

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## Keywords

Baikal natural territory, mtDNA, Siberian fir, bark beetle, population characteristics

## Introduction

The four-eyed fir bark beetle (FFBB) *Polygraphus proximus* Blandford, 1894 (Coleoptera: Curculionidae, Scolytinae) is invasive stem pest of Far Eastern origin. The secondary range of *P. proximus* in Siberia currently covers the territory of eight subjects namely Tomsk, Novosibirsk, Kemerovo Provinces, Republic of Altai, Altai and Krasnoyarsk krai, Republic of Khakassia and Irkutsk Province (Krivets et al. 2015; Bystrov, Antonov 2019). In the past decade FFBB has caused a large-scale dieback of Siberian fir *Abies sibirica* Ledeb in all invasion regions. Importantly, that Siberian fir is one of the key tree species in the Siberian taiga (Baranchikov and Krivets 2010; Krivets et al. 2015).

We tried to find FFBB populations in the Khamar-Daban fir forests (Buryatia Republic), where previously in 2014 the alien pest was not detected (Forest Pathological Research, electronic resource). In 2017 FFBB was found in foothills of Khamar-Daban (territory of Irkutsk Province, near the boundary Republic of Buryatia) in the Baikal dark coniferous forests (Bystrov and Antonov 2019). Based on the dendrochronological analysis this invasion dates back to 2011–2014 (Bystrov et al. 2019; Efremenko et al. 2019). We assumed that FFBB could invade this region in the following years.

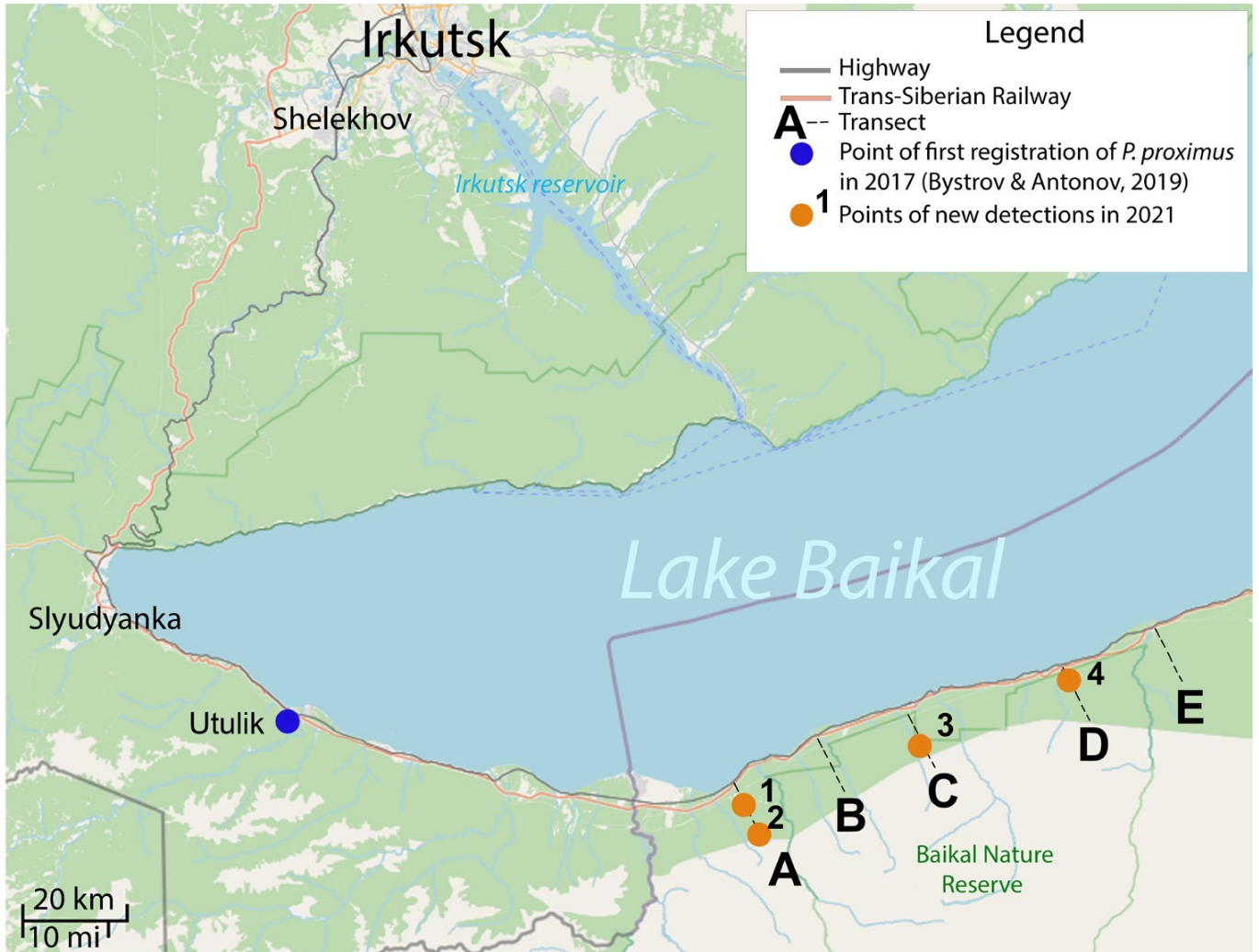
We have managed to do a forest pathology research of Khamar-Daban fir forests in south coast of Baikal Lake. Samples of FFBB were collected in different localities and mitochondrial DNA of adult insects was characterised in order to figure out the region-donor of invasion. There are five mitochondrial haplogroups of FFBB based on the nucleotide diversity of *COI* and *COII* genes (Kononov et al. 2016). In the original range the haplogroups I-IV of *P. proximus* are found in populations of Russian Far East, whereas the haplogroup V is found in Honshu Island (Kononov et al. 2016). In secondary range: haplogroup I was found in Novosibirsk, Tomsk, Kemerovo Provinces and Altai Republic, IV was found in Krasnoyarsk krai and Republic of Khakassia, and haplogroups II, III and V were not found in Siberia. Therefore, if we found haplogroups II, III or V it would mind that the source of invasion was native range. In case of haplogroups I or IV, the source of invasion could be as native as secondary range. It is important whether there were different haplogroups in a population, that means the source was from native range.

## Materials and methods

### Field studies and material collection

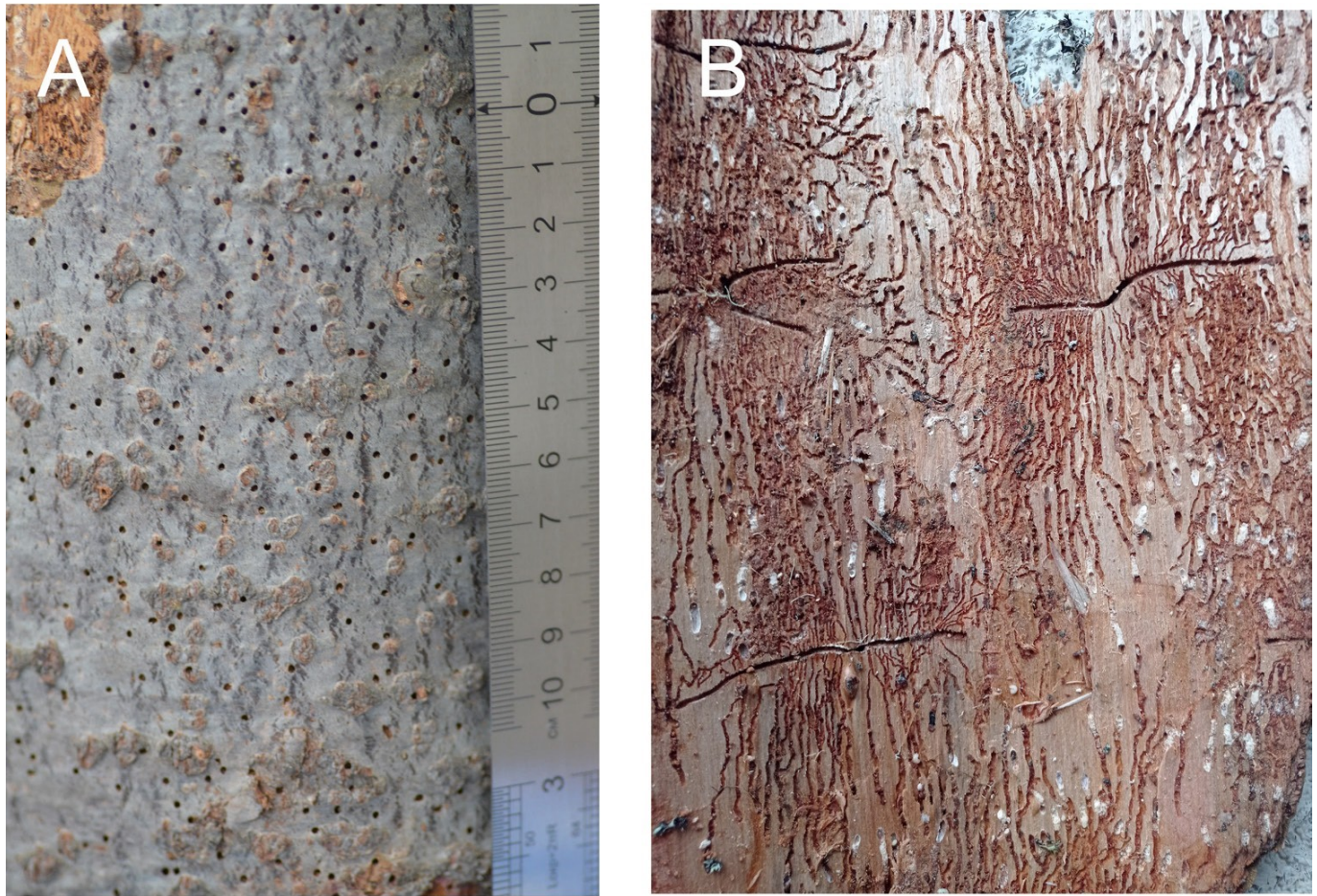
Four transects (A, B, D, E) along the mountain rivers flowing into Baikal Lake from the northern macroslope of the Khamar-Daban mountain range were inspected (Fig. 1). The routes were perpendicular to Trans-Siberian Railway line, each six km long and 40 m width; the average distance between adjacent transects was 20–25 km with 30 km. Studied area was at altitudes of 470–1130 m above sea level in a dark coniferous taiga belt represented by Siberian fir and Siberian pine (*Pinus sibirica* Du Roi) forests. All weakening and windfelled fir trees ( $\geq 200$  trees/transect) were examined in August 24–28 2021. We collected all life stages of FFBB, noted the coordinates of findings and FFBB activity on the trees such as borrow dust, entry and exit holes, galleries under the bark, pupal chambers deepened into sapwood (Fig. 2). Insect samples were stored in 96% ethanol for further molecular genetic studies. Primary study design included the fifth route (transect C), however it was not inspected in 2021 because of a bear activity in a period of the main

study. Nevertheless, we estimated this route based on photos of bark beetle galleries that were made in 2020 by a staff of the Baikal Nature Reserve (N.A. Belova).



**Figure 1.** The map of transects and FFBB colony findings in the dark coniferous forests of Khamar-Daban.





**Figure 2.** Signs of *P. proximus* tree colonization: **A** - exit holes in bark; **B** - maternal galleries with egg chambers and larval tunnels extending from them.

The population characteristics of revealed FFBB colonies were analyzed according to the methodological recommendations for determining its demographic indicators in invasive populations (Krivets et al. 2018). In particular, the characteristics were as follows: occurrence is the ratio of the number of trees with signs of colonization by *P. proximus* to the percentage of dying and dead recorded trees. Production is the number of young beetles hatched per 1 dm<sup>2</sup> of the trunk lateral surface (<20.1 individuals/dm<sup>2</sup> is low production; 20.1-30 is medium; >30 is high). Population density is the number of families per unit surface of the fodder substrate (< 3.1 nests/dm<sup>2</sup> is low density; 3.1-5.0 is medium; > 5.1 is high). Population growth rate is the ratio of the offspring individuals to the number of individuals of parental generation (<1.1 is low rate; 1.1-3 is medium; > 3.1 is high). The average length of the maternal gallery and the average fecundity (number of eggs/ female) were also measured.

## Genetic analysis of insects

We extracted DNA individually from 5 specimens collected in three localities. The specimens were homogenized in 200µL of SDS extraction buffer (10 mM Tris-HCl (pH 8.0), 25 mM EDTA, 0.5% SDS, 0.1 M NaCl) and incubated at 56°C for 1h. After purification with KOAc and precipitation total DNA was dissolved in 100 µL of the double-distilled water.

For all samples the barcoding region of the *COI* gene was amplified with primer set HCO2198/LCO1490 (Folmer et al., 1994). The PCR reaction was performed using BioMaster HS-Taq PCR (2×) (BiolabMix, Novosibirsk, Russia) with a 20 µL volume. The thermocycler protocol was 95°C for 5 min, followed by 35 cycles at 95°C for 15 s, 55°C 1 min, and 72°C for 40 s, plus a final

elongation step of 72°C for 3 min.

A design of our study was to sequence the 620 bp barcoding region of *COI* locus of samples collected in different sites and according these data to define haplogroups. It allows clearly identify haplogroups I, II and IV (Table 1) whereas identification of haplogroups III and V demands to use complete *COI* and *COII* locus. The amplicons were purified with exonuclease (ExoI) enzyme (New England Biolabs), and sequenced using BrilliantDye Terminator sequencing kit (Nimagen). Sequences were analyzed using FinchTV v. 1.4.0 (Geospiza Inc., Seattle, WA, USA) and deposited in the GenBank database under accession numbers OP558940 and OP558941. The alignment of sequences and phylogenetic analysis were performed in MEGA6.0 (Tamura et al. 2013) using Tamura 3-parameter model and bootstrap analysis with 1000 replicates.

Haplogroup	SNP position according to KR817585.1					
	1 8 5	2 3 9	2 9 0	3 9 5	5 2 4	5 9 3
I	A	G	A	G	G	G
II	G	G	A	G	A	A/G
IV	A	A	G	A	A	G
III_V	A	G	A	A	A	G

**Table 1.** Diagnostic SNPs for determination of haplogroups I, II, IV and III\_V of the 620 bp barcoding region of the *COI* gene of *P. proximus*

## Result

Adults, pupae and larvae of FFBB were found in two out of five transects in particular in transect A – valley of the Mamai River that was 60 km far from the site of the first report of FFBB (village Utylik) provided by Bystrov et al (2019), and transect D in the valley of Osinovka Mishikhinskaya River (Table 2).

The highest site where *P. proximus* found was the slope of the valley of the Bolshoi Mamai River in locality 2 at an altitude of 1030 m above sea level (Fig. 3).

The analysis of FFBB colonies clearly indicated the initial stage of the pest expansion in the Khamar-Daban dark coniferous taiga. In two out of three localities we registered low value of the occurrence FFBB and high value of the population growth rate (Table 2). Here the population density of *P. proximus* was rather low.

Thus we noted 2% of the species occurrence for windfelled trees, and 1% for standing trees i.e. many potentially vulnerable trees remained uncolonized. On the locality 1 (Bolshoi Mamai River) the FFBB completely colonized the windfelled tree with high density in 2021 (Table 2). The decomposition stage of windfelled tree here bearing galleries of single nest of FFBB indicates the end of summer 2019 – spring 2020 period of colonization.

Number of locality (coordinates DD); transect; year of the tree death	Indicators					
	Occurrence, % of total tree mortality caused by FFBB	Population density, families/dm 2	Production, individuals/ dm 2 of the lateral trunk surface	Population growth rate	Average fecundity, eggs/female	Average length of the egg gallery per family, cm
1 (51.415833;10 4.821667); A; 2020	1	1	44	22	75; 45–81	6.5; 4.5–7.5
2 (51.395276;10 4.845751); A; 2021	2	2.5; 1.5–4.2	33; 24–50	6.6	71; 46–91	4.8; 2–10.6
3 (51.563611;10	0.5	0.1	1.4; 0.8–2	26	53.3; 31–72	6.1; 2.5–8.6

5.394722); D; 2021						
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**Table 2.** Demographic indicators of FFBB at monitoring sites in the forests of Khamar-Daban (median values)

We tried to estimate the timing of the *P. proximus* origin and the rate of invasion in the Baikal region. Taking into account the dendrochronological analysis of the material obtained in 2014 by Bystrov et al (2019), as well as the forest pathology research of Khamar-Daban in 2014 by Kerchev (see the report of Nature communications of Baikal reserve) and data of this study we came to the conclusion that FFBB population in the Baikal fir forests most likely had originated not later than 2014. The average invasion speed of FFBB over 7 years was not less than 13.6 km/year that was based on an assumption that the eastern boundary of the invasive range had been extending along the most distant site at the transect D, i.e. the Osinovka Mishikhinskaya River from the area where it was first detected in Irkutsk region (Bystrov and Antonov 2019).

Genetic analysis was performed for five FFBB individuals that were collected from three colonies. For two samples, one from transect A and one from transect B, sequences were assembled from both forward and reverse primers and they were used for phylogenetic analysis. These samples belonged to haplogroup I of mtDNA that was widely distributed in the west of secondary FFBB range (Table 1, Figure 4).

Other samples, two from transect B and one from transect A, were analysed based on one direction sequencing (LCO1490 primer) and they were also belong to haplogroup I according to diagnostic SNPs (Table 1).





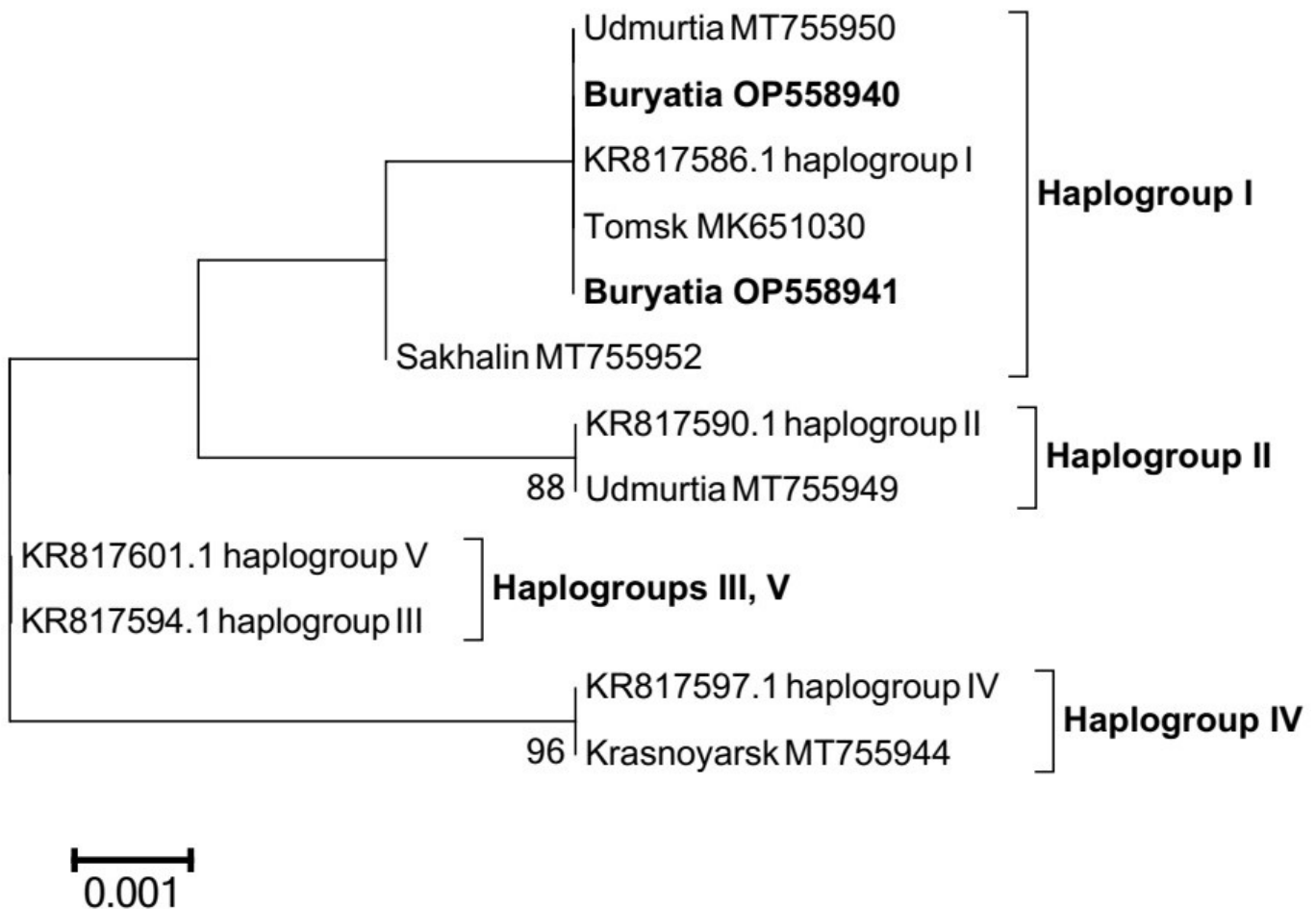


**Figure 3.** Infested Siberian fir tree on the slope of Mount Mamai (transect A).

## Discussion

This is the first report of *P. proximus* in the coniferous forests in Republic of Buryatia and the eastern area of the secondary range of FFBB.

The source of the *P. proximus* invasion in Khamar-Daban taiga is one of the crucial issue. Our results of mtDNA analysis indicated that most probably FFBB came from the secondary range, because we observed only one mtDNA haplotype that was widely distributed in the secondary range (Kononov et al. 2016; Bykov et al. 2020). Haplotype I was considered as dominant variant in the Tomsk and Kemerovo Provinces and Altay Republic whereas haplotype IV was dominant in Krasnoyarsk krai and Republic of Khakasia. It is important that there is disjunction of Siberian fir range between Krasnoyarsk krai and Byuriatia Republic. Therefore, we can exclude natural dispersal of FFBB from the populations of the secondary range. The invasion most probably concerned with economic activity such as railway transporting unbarked wood or other goods. During 2010–2015 the large scale outbreaks of *P. proximus* were noted in fir forests along railway in Tomsk and Kemerovo regions (Krivets et al. 2015). Therefore, we assume that a region-donor of invasion was Kemerovo or Tomsk Provinces. Moreover, these regions were characterized by high population densities of FFBB and they could appear the effect of propagule pressure during presumable date of invasion (Bystrov et al. 2019; Demidko and Efimov 2019). The general weakening and disturbance of local forests that was caused by the long-term anthropogenic impact, phytopathogens, and climate changes (Kharuk et al. 2016) could contribute to the success of the bark beetle population in a new territory.

**Figure 4.** ML phylogenetic tree of *P. proximus* was reconstructed with Tamura 3-parameter model of nucleotide



replacement based on sequences of 620 bp region of COI gene. Gen-Bank accession numbers and bootstrap values higher than 75 (1000 replicates) are indicated. Samples from this study are indicated in bold. Haplogroups of mtDNA are indicated according to Kononov et al. (2016).

We estimated the spread rate of FFBB on the Khamar-Daban coniferous forests as 13.6 km per year which was close to the values of bark beetle species of *Dendroctonus* Erichson, 1836 and *Ips* De Geer, 1775 genera (Jones et al. 2019). The absence of specific galleries on trees dried out more than 2–3 years ago on all inspected transects indicated that the spread rates could be several times greater than we calculated. The emergence of such dispersed small colonies could also be explained by the anemochory (spreading by wind) for example from the Utulik focus that was reported by Bystrov and Antonov (2019).

The altitude *P. proximus* colony in locality 2 was highest finding in this area. We assume FFBB can inhabit 1700 m in Siberian pine-fir woodlands as it observed in Mountain Shoria (Kemerovo province), where bark beetle was found on a host tree up to the altitudinal limit of *A. sibirica* distribution (Krivets et al. 2018).

The population indicators of *P. proximus* at all localities in Khamar-Daban suggest that alien pest has not yet fully realized its potential to colonize vulnerable trees due to recentness of its invasion. The production values were close to the FFBB maximum (Krivets et al. 2018) and the population density was below average, which indicated the initial stage of the population growth. The high rate of population growth and legislative ban on forest pest management measures on territories of Baikal nature preserve and water protection zone of the northern slope of Khamar-Daban allow us to expect bark beetle simultaneous outbreaks at a wide area in this region.

## Conclusion

The FFBB detection in the Khamar-Daban taiga is the first record of the alien pest in the Republic of Buryatia, and its locality was close to the east border range of Siberian fir, its main host-tree in the FFBB invasion territory. The molecular genetic analysis showed that the West Siberian bark beetle populations were the most probable donor region for invasion into the Baikal fir stands. FFBB populations were at the stage of the lag-phase of invasion however we noted high production that indicated a potential pest outbreak in the follow years. These data are important for more accurate forest conservation zoning and for proper planning of forest pathology monitoring.

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