Progress and plan towards eradication of the Non-native flies in King George Island, South Shetland Islands

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**Information Paper submitted by the Chile, Republic of Korea, Russian Federation, Uruguay**

***Summary***

Non-native flies (NNF, *Trichocera maculipennis*) have colonised several station sewage treatment plants on King George Island, South Shetland Islands. Chile, Republic of Korea, Russian Federation and Uruguay would provide to the Parties our achievement, progress of international collaboration up to now and future plans to develop effective NNF eradication manual.

To assess the genetic variability of NNF populations in King George Island (KGI), DNA barcode fragments of mitochondrial DNA COI and 12 polymorphic microsatellite loci were used. Based on the analyses, we assume that there were at least two separate NNF introduction events from different localities. Despite the low levels of diversity and the geographically short distances between the stations in KGI, the microsatellite results suggest the presence of four genetically distinct populations.

We tried to ascertain if there are established populations of NNF in natural ecosystems of KGI and surrounding islets. For this purpose, twelve different-type organic-rich wet habitats (potentially most attractive for NNF and suitable for its larvae development) situated in ten localities were examined for the presence of NNF immatures or traces/remains of NNF. No data was found testifying to the presence of NNF populations in natural Antarctic environment. We also used environmental DNA (eDNA) to identify the traces of it in the ecosystems. Freshwater and sediment samples from KGI were examined to test the presence of NNF DNA fragments. So far, no positive sign has been detected.

To understand the communication system of NNF, we examined the olfactory and visual sensory systems of NNF. The electron microscope observations and electrophysiological recordings indicate that NNF has sensitive and selective olfactory sensory system, suggesting that olfactory communication is important in NNF’s main activities such as mating and oviposition. The electroretinnogram recordings have also indicated that adult NNF’s well-developed compound eyes are highly sensitive to green and blue lights as well as UV light. We will complete an eradication manual for NNF by development of species-specific attractant combining olfactory and visual sensory cues, pesticide impregnated netting, and environmentally sound tools to eliminate NNF larvae. To prevent further introduction and spread of NNF in KGI, we urgently need to establish unique source localities and pathways of introduction, as well as extend our monitoring program in collaboration with all stations and facilities to develop successful NNF management strategies.

***Background***

Within the past decade there have been reports of sewage treatment plant infestations by the boreal trichocerid fly *Trichocera maculipennis* at research stations around Maxwell Bay (Guardia Nacional/Fildes Bay), King George Island/Isla 25 de Mayo, all of which lie within 10 km of one another (ATCM XXXIX – WP52). The Republic of Korea, United Kingdom, Chile and Uruguay recommended that the CEP promoted a co-ordinated international response by encouraging Parties with stations on King George Island to check their sewage treatment plants for non-native invertebrate infestations and, if present, join collaborative research to identify and determine the origin of these species.

As the first step of a co-ordinated international response to manage this non-native fly (NNF), in 2017 most of the overwintering stations on King George Island/Isla 25 de Mayo checked their station buildings, facilities, and surrounding areas in response to the survey entitled ‘A short questionnaire on Non-native Flies at Antarctic Stations’. Based on the findings of this survey, the Republic of Korea, Uruguay, Chile, and the United Kingdom recommend that the CEP: (1) continues to encourage Parties with stations on King George Island/Isla 25 de Mayo to check their facilities for non-native flies and to undertake both continuous and periodic monitoring to indicate, if there are any non-native flies inhabiting the environment; (2) encourages the Parties to jointly develop co-ordinated standardized monitoring and eradication programmes to effectively control the spread of the flies, and join the collaborative research project; and (3) asks COMNAP to play a central role in sharing information and best practices between Parties and other stakeholders (ATCM XL – WP26). According to that survey, *T. maculipennis* had been recorded within or in the surroundings of the following stations: Artigas, Arctowski, Escudero, Frei, Fildes and King Sejong, without further confirmation of the presence or absence of the species in the other stations of King George Island/Isla 25 de Mayo.

In the Antarctic summer season 2017/2018 Uruguay, the Republic of Korea, Chile and the Russian Federation started a pilot monitoring programme with support from Germany, to track and avoid the expansion of *T. maculipennis* in King George Island/Isla 25 de Mayo (ATCM XLI – IP50). The coordinated actions implemented were installation of 1) adhesive traps inside the facilities (Artigas, Bellingshausen, Fildes and King Sejong stations) to identify population trends throughout the year, and 2) pitfall traps in the surroundings of Artigas and King Sejong stations during the summer season to assess the presence of the species beyond the limits of these stations.

In the 2018/2019 season Argentina, Brazil and China joined the monitoring programme. Adhesive traps installed in Artigas, Bellingshausen, Carlini, Escudero, Ferraz, Great Wall and King Sejong stations. During the summer season, pitfall traps were installed in the surroundings of stations at Fildes Peninsula and water samples of several lakes were taken to perform analyses using environmental DNA techniques. Genetic analyses were also performed to assess population structure from individuals collected in Artigas, Escudero and King Sejong stations (ATCM XLII – IP120).

In the 2019/2020 season the Russian Federation started research to locate larval habitats of *T. maculipennis* in the anthropogenic habitats (indoors and outdoors) located in the territory of Bellingshausen station and to assess presence of this species immatures in selected natural habitats on Fildes Peninsula, King George Island and on surrounding islets. In addition, that season Peru, Poland and Spain joined the joint monitoring, installing sticky traps in Arctowski and Machu Picchu (King George Island/Isla 25 de Mayo), Gabriel de Castilla (Deception Island) and Juan Carlos I (Livingston Island) stations.

We would provide to the Parties our achievement and progress of international collaboration and plans to develop effective NNF eradication manual.

***Genetic variability analyses of NNF***

Identifying the origin (source locality) of NNF is a crucial step in designing management strategy, and the level of genetic variation is a key factor influencing the longevity and adaptive potential of founder individuals (Lee, 2002; Genton et al., 2006). To assess the genetic variability of NNF populations in King George Island, DNA barcode fragments of mitochondrial DNA (mtDNA) COI (cytochrome *c* oxidase subunit I) and 12 polymorphic microsatellite loci were used.

For the mtDNA (COI) analysis, we analysed 10 NNF individuals taken from four stations (Frei, Bellingshausen, Artigas, King Sejong) during 2017/2018 - 2019/2020 austral summer seasons and one individual of the same species from Spitsbergen, Svalbard Islands in the Arctic. Three COI sequences including the sequence from Polish Arctowski station uploaded at NCBI were analysed together. As the result, two distinct lineages were identified from the mtDNA analysis. Lineage I is associated with the Arctic and Polish sequences, while Lineage II is associated with the Northern American. The genetic variation (3%) existing between two lineages indicates possible population structures in native ranges and multiple introductions of NNF from at least two different origins (Annex Figure 1). A total of five haplotypes are examined and distribution varies depending on the stations (Annex Figure 2). Only Chilean Frei base is occupied by both lineages, which is anticipated in terms of its position as a transport hub in King George Island (Potocka and Krzemińska 2018). Based on mtDNA analysis, we assume there were at least two separate NNF introduction events from different localities. Annex provides further details with figures.

Microsatellite variation was investigated in five populations from the stations (Artigas, Bellingshausen, Escudero, Frei, King Sejong). Microsatellites provide more power to discern population structure even in low genetic diversity due to their high mutation rate. Despite the low levels of diversity and the geographically short distances between the stations, the result shows the evidence of 4 genetically distinct populations (Annex Figure 3).

The founder effect, in which a small number of flies settle to form a new population in each station while losing genetic diversity from the original population, explains this phenomenon. Its reproduction has been reliant on isolated sewage treatment facilities, and as a result of the factor, there has been substantial population differentiation in each station. The results of genetic analyses are being prepared for publication.

***Possibility of survival of NNF in Antarctic environment***

Although there is no indication that NNF completes its life cycle under outdoor conditions in King George Island, future successful expansion of the cold-tolerant NNF into terrestrial and semiaquatic organic-rich natural habitats cannot be excluded with global warming. *T. maculipennis* is already a persistent non-native species in the natural environment of some sub-Antarctic islands (Frenot et al., 2005).

The establishment of NNF in natural Antarctic habitats could be catastrophic as saprophagous larvae can alter the existing environments. The analysis of the published data on life history and larval habitats of *T. maculipennis* (NNF), other species of Trichoceridae, and own observations on anthropogenic larval habitats of NNF on KGI enabled us to suppose that future successful expansion of NNF into natural Antarctic environments is possible. According to existing knowledge, the larvae are saprophagous, i.e. they feed on dead organic matter. The larval habitats include terrestrial wet and semiaquatic organic-rich substrata, which can be from wet to semiliquid.

**Studies by Republic of Korea**

The multi-year monitoring of King Sejong station indicates that the occurrence of NNF has been persistent with two adult peaks each year. Currently, the distribution of NNF appears to be limited to the station, and we do not yet have any evidence that larval breeding takes place elsewhere except two sewage treatment facilities in the station. However, indoor and outdoor behavioral tests indicate that the edge of freshwater lakes around the station can be an ideal place for the oviposition and pre- and post-embryonic developments of NNF, once temperature condition becomes favourable. Female NNF prefer laying eggs in the lake water, and NNF larvae actively feed on various organic matters in the edge of freshwater lake. In contrast, the predation of young NNF larvae by native arthropods such as springtails and midges were observed, and parasitism by microscopic organisms present in the freshwater was also found. Although long larval period, and predation and parasitism by highly populated native organisms make it less likely that NNF completes its life cycle in the nearby freshwater lakes, more information is needed regarding potential survival of NNF in Antarctic environment. At present, the most limiting factor for NNF’s outdoor survival appears to be the long larval period that would make it difficult to grow to pupae before the lake gets frozen.

**Studies by Russian Federation**

The laboratory tests conducted on KGI in the 2019/2020 season confirmed that the larvae of NNF are able to complete development in organic-rich substrata taken from natural habitats of KGI.

In the same season, we tried to ascertain if established populations of NNF already existed in natural ecosystems of KGI (Fildes Peninsula) and surrounding islets. For this purpose, twelve different-type organic-rich wet habitats situated in ten localities were examined for the presence of NNF immatures or traces/remains of NNF. We have chosen the habitats potentially most attractive for NNF and suitable for its larvae development, according to the published data on life history and larval habitats of NNF and own observations on larval bionomics of NNF. The habitats examined were most similar to the known natural (boreal) habitats of this species, as well as to the few known anthropogenic habitats of this species in the Antarctic. Four sites examined were situated near Bellingshausen station, while the other sites were at a distance of several kilometers from any station. In all habitats and localities, no specimens of NNF were found. No remains of larvae, pupae or adults were found either. Hence, we can preliminarily conclude that so far no evidence has been found for NNF development in natural habitats of King George Island, and for colonization of Antarctic ecosystems by NNF.

**Studies by Uruguay**

The multi-year monitoring of Artigas shows a persistent occurrence of NNF, with higher numbers of individuals captured during the summer season. 93% of total captures were recorded in the septic tank of the staff accommodation building.

Through the use of pitfall traps around Artigas station, some individuals have been recorded outside the station; however no larvae presence or colonisation of environments outside the buildings has been detected.

As an additional control measure to the UV traps, in the 2019/2020 season, an air fan extractor with a mesh at the free end was installed at the site with the most individuals to retain the sucked individuals. As reported by station staff, this system is very effective, capturing a high number of individuals. Due to the pandemic, data from last season's adhesive traps could not be processed, so the evaluation of this new measure based on statistical data is not yet available.

Furthermore, the pandemic situation prevented the start of experiments on eco-physiology and morphology of the species, planned to be carried out in Artigas during the summer season 2020/2021, which are expected to be carried out next season.

***Environmental DNA analysis***

Identifying the presence and distribution of invasive species is critical for invasive species research, and species detection methods based on environmental DNA (eDNA) have emerged as new monitoring tools (Goldberg *et al*., 2016). We examined freshwater and sediment samples from King George Island to test the presence of NNF DNA fragments. So far, no positive result has been reported but we must continue to monitor the situation. Because of global warming and the growing possibility of NNF persisting outside of stations, continuous and frequent monitoring of the occurrence of NNF in the natural environment is needed.

***Progress of eradication programme***

The study at different research stations in King George Island indicates that UV traps are much more attractive to NNF adults than delta traps with white adhesive bases or white adhesive trap alone. It also shows that the conventional UV light bulbs are more attractive to NNF than UV LEDs. Although UV traps seem to be suitable for the monitoring of NNF, however, more powerful attractant and more convenient traps would be needed for eradication programme since effective lure-and-kill and delimitation with potent attractant is essential in successful eradication. Currently, UV traps require electricity for UV light and electrical fan, which limits trap placement. Species-specific odour bait can be an ideal attractant, alone or in combination with visual cues, in eradication programme, as shown in a number of successful eradications of invasive insects.

The electron microscope observations and electrophysiological recordings indicate that NNF has sensitive and selective olfactory sensory system, suggesting that olfactory communication is important in NNF’s main activities such as mating and oviposition. Based on this, we have initiated our effort to develop odour-based attractants for NNF: headspace samples have been prepared, electroantennogram recording system constructed, and odour-baited trapping system established at the King Sejong station. In the preliminary study using this system, it has been found that 1) NNF adults have well developed olfactory sensilla on the antennae and maxillary palps, 2) the antennae of NNF are specialized for detecting a narrow range of volatile compounds, and 3) the headspace samples collected from the sewage treatment facility contain highly olfactory active compounds for NNF. The electroretinnogram recordings have also indicated that adult NNF’s well-developed compound eyes are highly sensitive to green and blue lights as well as UV light.

In the 2019/2020 season the larval habitats and larval populations were found on Bellingshausen station. The larvae were found only indoors, in a single building. No larvae, pupae or their remains were found in anthropogenically affected habitats outdoors. The recommendations have been formulated for eradication of existing larval populations and preventive measures for their re-establishment in future. We suppose that absence of available indoor larval habitats is critical for successful eradication of existing populations of *T. maculipennis*.

***Future plans***

Over the next few years, we will complete an eradication manual for NNF and initiate eradication practice according to the manual. To complete the eradication manual,

* new attractant will be developed by combining olfactory and visual sensory cues,
* pesticide-impregnated netting will be prepared to prevent the movement of adult NNF between inside and outside of sewage treatment facility, and
* environmentally sound tools will be introduced to eliminate NNF larvae in the sewage treatment facility.

To develop new attractants, olfactory-active compounds will first be identified, then the chemical structure of the olfactory-active compounds will be elucidated by chemical analysis. Attractiveness of the olfactory-active compounds will be evaluated through trapping tests individually and in combination.

Pesticide-impregnated netting will be carefully selected and designed so that the pesticide does not spill over to the environment while maintaining effective contact-toxicity for NNF adults, for which necessary parameters can be measured using lab-reared and field-collected colonies of NNF or related species.

While adult NNF are managed, NNF larvae present inside the sewage treating tanks will be removed by using non-toxic means such as targeted spray of hot water, suffocating larvae with vegetable oil, and ultrasonic treatment. The effectiveness of these tools has been yet to be evaluated over the next couple of years.

To prevent further introduction and spread of NNF in King George Island, we urgently need to establish unique source localities and pathways of introduction, as well as extend our monitoring program in collaboration with all stations and facilities in King George Island to develop successful NNF management strategies.

***Supporting documentation***

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