NDH gene disruption – possible implication for adaptation of *Allium* species to different light conditions

A.S. Speranskaya^{1, 8*}, M.S. Belenikin², E.A. Konorov^{3, 4}, S.V. Kuptsov¹, M.I. Antipin¹, M.D. Logacheva⁵, D.O. Omelchenko^{1, 6}, A.A. Krinitsina^{1, 7}

¹ Lomonosov Moscow State University, Moscow, Russia

² Moscow Institute of Physics and Technology, Dolgoprudny, Russia

³ Vavilov Institute of General Genetics of Russian Academy of Sciences, Moscow, Russia

⁴ V.M. Gorbatov Federal Research Center for Food Systems of Russian Academy of Sciences, Moscow, Russia

⁵ Skolkovo Institute of Science and Technology. Russia, Moscow Region

⁶ Institute for Information Transmission Problems, Moscow, Russia

⁷ All-Russia Research Institute of Agricultural Biotechnology, Moscow, Russia

⁸ I.M. Sechenov First Moscow State Medical University of the Russian Ministry of Health (Sechenov University), Department of Medical Genetics, Moscow, Russia

DOI 10.18699/ICG-PlantGen2019-55	Abstract: We have analyzed plastome sequences of twelve <i>Allium</i> species and found certain features reflecting evolutionary processes in the genus. The <i>A. paradoxum</i> plastome
© Autors, 2019	sequence differed markedly from other <i>Allium</i> species by a large 4,825 bp long inversion in the SSC region and pseudogenization of all genes encoding the NADH complex. The NADH
* e-mail: hanna.s.939@gmail.com	complex is presumably required to optimize photosynthesis. In <i>ndh</i> defective plants the photosynthesis rate decreases under excess light. We assume that the natural distribution of <i>A. paradoxum</i> , restricted to shady forests, is linked to <i>ndh</i> genes' impairment. No other analyzed <i>Allium</i> species demonstrated these features, not even the phylogenetically close shade-tolerant <i>A. ursinum</i> .
	Key words: Allium; plastome; shaded environments; adaptation; NDH genes; gene loss; pseudogenization.

Finding genetic markers that reflect adaptation of plants to certain ecological niches is an important task with many possible practical outcomes. This field of plant molecular ecology is vast. Modern methods of genetic analysis applied to plants proved fruitful in solving many ecological problems: findings such as conditional expression and methylation changes led to insights into the genetic basis of adaptation to many stress factors: drought (Cortés et al., 2012; Shi et al., 2013; Rico et al., 2014), herbivorous insects (Smith, Clement, 2012; Eyres et al., 2016), cold hardiness (Gusta, Wisniewski, 2013), salinity (Hasegawa, 2013), soil acidity (Kochian et al., 2015) and others.

Light intensity is one of the most important abiotic factors in plant life. Plants mostly growing in shady habitats can be either shade-loving (sciophilous) or just shade-tolerant. Shadetolerant plants, or scioheliophytes, can cope with low light levels but are also able to grow in more or less open habitats with some degree of direct sunlight, while shade-loving plants stunt their growth or get sunburns if exposed to direct sun.

It is not yet fully known what genetic factors determine the development of shade tolerance, but certain data suggest that these traits may be inherited and are under natural selection. In the recently sequenced Panax ginseng genome, there is an amplification of chlorophyll a/b proteins (CAB), which is proposed to be a shade adaptation (Kim et al., 2018). There are also works that show some genetic basis for shade-loving and shade-tolerance predisposition in tomato (Sulistyowati et al., 2016; Ritonga et al., 2018).

Species in the genus *Allium* are adapted to habitats with a wide range of light intensity, from shady forests to open habitats like meadows, steppes and deserts. We have analyzed plastome sequences of twelve wild and cultivated *Allium* species. Ten of them, namely *A. ursinum*, *A. paradoxum* (MH053150), *A. macleanii*, *A. nutans*, *A. platyspathum*, *A. schoenoprasum*, *A. pskemense*, *A. obliquum* (MH157875), *A. fistulosum*, *A. victorialis* were sequenced, assembled and annotated in our lab. Sequences for *A. cepa* (KF728080) and *A. sativum* (KX683282) were obtained from works of von Kohn et al. (2013) and Filyushin et al. (2016).

A wealth of data regarding higher plants' plastide genomes is available at present. Full plastomes of more than 8000 plant species have been currently analyzed, of which more than 10 belong to the genus *Allium* (for several *Allium* species, no final annotations have been published yet and we are not analyzing them in this work). The plastomes of all studied *Allium* species contain the same number of tRNA genes (30 in total, of them 9 are represented by two copies in IR) and rRNA (8 genes, all 4 are represented by two copies in IR), 79 genes encode proteins. In general, the *Allium* plastomes analyzed had similar numbers, arrangement and orientation of genes (data not published). Yet some of the analyzed species proved to have deletions of certain genes, e.g. the small ribosome subunit gene *rps16* (Belenikin et al., 2016).

The *A. paradoxum* plastome was quite distinct from other species in that all its *ndh* genes were subject either to deletion or to pseudogenization. It also had a large (4825 kB) inversion in the SSC region. A number of other distinctions in the plastome structure of the species have been identified (Omelchenko et al., 2019, in press). All other analyzed *Allium* species did not have any alterations in the structure of the *ndh* gene family. The NADH-dehydrogenase complex protects plants in stress conditions, preventing oxidative stress, partici-

pating in electron cyclic transport and chloroplast respiration at night. It is divided into five subcomplexes, of which only one, the membrane subcomplex, is encoded by plastome genes only, while the others are encoded by nuclear or both nuclear and plastome genes. Genes of the NADH-dehydrogenase complex are also found in the mitochondrial genome, but they are of a different origin compared to plastome ones (according to Onoiko, Zolotareva, 2014). The proteins of the membrane NADH-dehydrogenase complex in the plastome are encoded by 11 ndh genes (ndhA-K). Deletion of different plastome genes is sometimes accompanied by their transfer to the mitochondrial or nuclear genome (Logacheva et al., 2016; Lin et al., 2017). In some representatives of Pinaceae (Picea abies and Pinus massoniana), non-functioning fragments of ndh genes were found in the nuclear genome (Ranade et al., 2016; Ni et al., 2017), and in several Orchidaceae, their copies were found in mitochondrial genomes (Lin et al., 2015). Deletion or pseudogenization of plastome ndh genes is not always accompanied by their copies appearing elsewhere, e.g. in many orchids, despite the loss of *ndh* plastome genes, they were not found in their nuclear genome either (Cai, 2015; Lin et al., 2017). Ruhlman et al. (2015) have analyzed transcriptomes of both flowering plants and gymnosperms (Pinaceae, Gnetales, Geraniaceae and Orchidaceae) that had lost plastome *ndh* genes and did not found any functional copies of these genes.

In a considerable number of plant species belonging to unrelated taxonomic groups some *ndh* genes are deleted, while the others had become pseudogenes (e.g. Pinaceae (Ranade et al., 2016; Ni et al., 2017), Gnetales (Ranade et al., 2016), Orchidaceae (Chang et al., 2005; Lin et al., 2017) and others). In all cases mentioned above, all *ndh* plastome genes had lost their function. It is possible that this deletion is linked to operon organization of plastome genes (Sanderson, 2015). In particular, *ndh* genes are also organized into an operon (Maier et al., 1995; del Campo et al., 2005). Loss of *ndh* genes is considered to be an ancient trait, common to all Pinaceae (Lin et al., 2010; Wu et al., 2011). On the other hand, deletions of *ndh* genes in Orchidaceae had most likely occurred independently in several taxonomic lines in the family (Lin et al., 2015).

Considering that many unrelated taxa had lost their plastome *ndh* genes independently, we can speculate that this feature is a consequence of convergent evolution. An indirect confirmation of *ndh* gene defunctionalization being an adaptive event can be found in the fact that it occurs in plants as primitive as Marchantiophyta (Wickett et al., 2008).

Mutant *ndh* gene types are almost never affecting the phenotype if the plant is growing in its optimal conditions, but these plants are less stress tolerant. It is possible that electron cyclic transport is required for photosynthesis to be effective in stress conditions, such as drought, heat, high light intensity (Horvath et al., 2000; Wang et al., 2006; Sanderson et al., 2015; Yamori et al., 2015), and also in low light levels, as the NADH-dehydrogenase complex is known to mediate the minor pathway of electron cyclic transport in photosystem I. Analysis of ecological and trophic preferences of plants with *ndh* deletions has shown that it is common in plants with some degree of heterotrophy (Wicke et al., 2011; Petersen

et al., 2015; Silva et al., 2016, 2018; Shin, Lee, 2018). On the other hand, hemiparasitic plants do not always defunctionalize their *ndh* genes. All *ndh* genes are functional in the plastome of *Aureolaria virginica* belonging to Orobanchaceae, a family characterized by a different level of *ndh* gene pseudogenization (Frailey et al., 2018).

Nevertheless, a full loss of all *ndh* genes can be found not only in plants with various degrees of heterotrophy, but also in obligate autotrophic species. Besides *Allium paradoxum* it was found in some Pinaceae (Ni et al., 2017), in *Erodium* (Blazier et al., 2011), in the monotypic genera *Circaeaster* and *Kingdonia* (Circaeasteraceae), and even in Cactaceae in *Carnegiea gigantea* (Sanderson et al., 2015).

At least some events of *ndh* pseudogenization may be possibly linked to light intensity adaptations. As was mentioned before, a properly functioning NADH-dehydrogenase complex allows plants to adapt flexibly to light intensities that are far from optimal, while defects in it may influence plant reactions to both high (800 μ mol photons m⁻² s⁻¹) and low (200 μ mol photons m⁻² s⁻¹) light intensity (Yamori et al., 2015; Ruhlman et al., 2015).

As the loss of *ndh* genes makes intense light a stress factor, we suppose that *ndh* gene defunctionalization and shade-loving habit are linked in Allium paradoxum. All the species in the study that are related to A. paradoxum had a full functioning set of *ndh* genes. Its closest relative in the study, A. ursinum, is a representative of the same evolutionary line (first) and the same subgenus. On a phylogenetic tree, these species are in neighboring clades (Hanelt, 1996; Friesen, Fritsch, 2006). A. ursinum also prefers forest habitats, but it requires rather high light conditions, starting to grow before canopy closure, and its distribution is thought to be limited mostly by water availability, as it can grow successfully in open habitats if precipitation is high and evenly distributed or in the vicinity of rivers and streams (Oborny et al., 2011). Thus we can suppose that historically both understorey Allium species were shade-tolerant, but after the loss of functionality of its ndh genes A. paradoxum became a shade-loving species and lost the ability to grow in open habitats.

Nevertheless, several species with the defunctionalization of *ndh* genes grow in mesic or arid open habitats and do not suffer from intense light (*Carnegiea*, *Pinus*, *Circaeaster* and *Kingdonia*), and thus had supposedly developed a different mechanism of light stress tolerance than the NADH gene complex.

References

- Belenikin M.S., Krinitsina A.A., Logacheva M.D., Kuptsov C.V., Speranskaya A.S. The quest for evolutionary changes in plants adapted to high-altitude habitats: the next-generation sequencing and comparative analysis of chloroplast genomes of some Allium species. *Plant Protection News*. 2016;3(89):23–24.
- Blazier J.C., Guisinger M.M., Jansen R.K. Recent loss of plastid-encoded ndh genes within Erodium (Geraniaceae). *Plant Mol. Biol.* 2011;76(3–5):263–272. DOI 10.1007/s11103-011-9753-5.
- Cai J., Liu X., Vanneste K., Proost S., Tsai W.C., Liu K.W., Chen L.J., He Y., Xu Q., Bian C., Zheng Z., Sun F., Liu W., Hsiao Y.Y., Pan Z.J., Hsu C.C., Yang Y.P., Hsu Y.C., Chuang Y.C., Dievart A., Dufayard J.F., Xu X., Wang J.Y., Wang J., Xiao X.J., Zhao X.M., Du R., Zhang G.Q., Wang M., Su Y.Y., Xie G.C., Liu G.H., Li L.Q.,

Huang L.Q., Luo Y.B., Chen H.H., de Peer Y.V., Liu Z.J. The genome sequence of the orchid Phalaenopsis equestris. *Nat Gen.* 2015; 47(1):65. DOI 10.1038/ng.3149.

- Chang C.C., Lin H.C., Lin I.P., Chow T.Y., Chen H.H., Chen W.H., Cheng C.H., Lin C.Y., Liu S.M., Chang C.C., Chaw S.M. The chloroplast genome of Phalaenopsis aphrodite (Orchidaceae): comparative analysis of evolutionary rate with that of grasses and its phylogenetic implications. *Mol. Biol. Evol.* 2005;23(2):279–291. DOI 10.1093/molbev/msj029.
- Cortés A.J., Chavarro M.C., Madriñán S., This D., Blair M.W. Molecular ecology and selection in the drought-related Asr gene polymorphisms in wild and cultivated common bean (*Phaseolus vulgaris* L.). *BMC Gen.* 2012;13(1):58. DOI 10.1186/1471-2156-13-58.
- del Campo E.M., Sabater B., Martín M. Characterization of the 5'-and 3'-ends of mRNAs of ndhH, ndhA and ndhI genes of the plastid ndhH-D operon. *Biochimie*. 2006;88(3–4):347–357. DOI 10.1016/j. biochi.2005.09.005.
- Eyres I., Jaquiéry J., Sugio A., Duvaux L., Gharbi K., Zhou J.J., Legeai F., Nelson M., Simon J.-C., Smadja C.M., Butlin R., Ferrari J. Differential gene expression according to race and host plant in the pea aphid. *Mol Ecol.* 2016;25(17):4197–4215. DOI 10.1111/mec. 13771.
- Filyushin M.A., Beletsky A.V., Mazur A.M., Kochieva E.Z. The complete plastid genome sequence of garlic *Allium sativum* L. *Mitochondrial DNA*. Part B. 2016;1(1):831–832. DOI 10.1080/23802359. 2016.1247669.
- Frailey D.C., Chaluvadi S.R., Vaughn J.N., Coatney C.G., Bennetzen J.L. Gene loss and genome rearrangement in the plastids of five Hemiparasites in the family Orobanchaceae. BMC Plant Biol. 2018; 18(1):30.
- Friesen N., Fritsch R.M., Blattner F.R. Phylogeny and new intrageneric classification of Allium (Alliaceae) based on nuclear ribosomal DNA ITS sequences. *Aliso: A J Systematic Evolutionary Bot.* 2006;22(1):372–395. DOI 10.5642/aliso.20062201.31.
- Gusta L.V., Wisniewski M. Understanding plant cold hardiness: an opinion. *Physiol Plantarum*. 2013;147(1):4–14. DOI 10.1111/j.1399-3054.2012.01611.x.
- Hanelt P. Taxonomic problems in Mediterranean Allium, and relationships with non-Mediterranean Allium groups. *Bocconea*. 1996;5(1): 259–265.
- Hasegawa P.M. Sodium (Na+) homeostasis and salt tolerance of plants. *Environmental Experimental Bot.* 2013;92:19–31. DOI 10.1016/j. envexpbot.2013.03.001.
- Horváth E.M., Peter S.O., Joët T., Rumeau D., Cournac L., Horváth G.V., Kavanagh T.A., Schäfer C., Peltier G., Medgyesy P. Targeted inactivation of the plastid ndhB gene in tobacco results in an enhanced sensitivity of photosynthesis to moderate stomatal closure. *Plant Physiol.* 2000;123(4):1337–1350. DOI 10.1104/pp.123. 4.1337.
- Kim N.H., Jayakodi M., Lee S.C., Choi B.S., Jang W., Lee J., Kim H.H., Waminal N.E., Lakshmanan M., Nguyen B., Lee Y.S., Park H.S., Koo H.J., Park L.Y., Perumal S., Joh H.J., Lee H., Kim J., Kim I.S., Kim K., Koduru L., Kang K.B., Sung S.H., Yu Y., Park D.S., Choi D., Seo E., Kim S., Kim Y.C., Hyun D.Y., Park Y.I., Kim C., Lee T.H., Kim H.U., Soh M.S., Lee Y., In J.G., Kim H.S., Kim Y.M., Yang D.C., Wing R.A., Lee D.Y., Paterson A.H., Yang T.J. Genome and evolution of the shade-requiring medicinal herb Panax ginseng. *Plant Biotechnol. J.* 2018;16(11):1904–1917. DOI 10.1111/ pbi.12926.
- Kochian L.V., Piñeros M.A., Liu J., Magalhaes J.V. Plant adaptation to acid soils: the molecular basis for crop aluminum resistance. *Annual Review Plant Biol.* 2015;66:571–598. DOI 10.1146/annurevarplant-043014-114822.
- Lin C.P., Huang J.P., Wu C.S., Hsu C.Y. Comparative chloroplast genomics reveals the evolution of Pinaceae genera and subfamilies. *Gen Biol Evolution*. 2010;2:504–517. DOI 10.1093/gbe/evq036.

- Lin C.S., Chen J.J., Chiu C. C., Hsiao H.C., Yang C.J., Jin X.H., Leebens-Mack J., de Pamphilis C.W., Huang Y.T., Yang L.H., Chang W.J., Kui L., Wong G.K.S., Hu J.M., Wang W., Shih M.C. Concomitant loss of NDH complex-related genes within chloroplast and nuclear genomes in some orchids. *Plant J.* 2017;90(5):994– 1006. DOI 10.1111/tpj.13525.
- Lin C.S., Chen J.J., Huang Y.T., Chan M.T., Daniell H., Chang W.J., Hsu C.T., Liao D.C., Wu F.H., Lin S.Y., Liao C.F., Deyholos M.K., Wong G.K.S., Albert V.A., Chou M.L., Chen C.Y., Shih M.C. The location and translocation of ndh genes of chloroplast origin in the Orchidaceae family. *Sci Reports*. 2015;5:9040. DOI 10.1038/ srep09040.
- Logacheva M.D., Schelkunov M.I., Shtratnikova V.Y., Matveeva M.V., Penin A.A. Comparative analysis of plastid genomes of non-photosynthetic Ericaceae and their photosynthetic relatives. *Sci Reports*. 2016;6:30042. DOI 10.1038/srep30042.
- Maier R.M., Neckermann K., Igloi G.L., Kössel H. Complete sequence of the maize chloroplast genome: gene content, hotspots of divergence and fine tuning of genetic information by transcript editing. *J Mol Boil*. 1995;251(5):614–628. DOI 10.1006/jmbi.1995.0460.
- Ni Z., Ye Y., Bai T., Xu M., Xu L.A. Complete chloroplast genome of Pinus massoniana (Pinaceae): Gene rearrangements, loss of ndh genes, and short inverted repeats contraction, expansion. *Molecules*. 2017;22(9):1528. DOI 10.3390/molecules22091528.
- Oborny B., Botta-Dukat Z., Rudolf K., Morschhauser T. Population ecology of Allium ursinum a space-monopolizing clonal plant. *Acta Botanica Hungarica*. 2011;53:371–388. DOI 10.1556/ABot.53. 2011.3–4.18.
- Onoiko E.B., Zolotareva E.K. The chloroplast NAD(p)H-dehydrogenase complex of the highest plants. *Plant Physiol Gen* (*Kyiv*) 2014; 46:371-384.
- Petersen G., Cuenca A., Seberg O. Plastome evolution in hemiparasitic mistletoes. *Genome Biol Evol*. 2015;7(9):2520–2532. DOI 10.1093/ gbe/evv165.
- Ranade S.S., García-Gil M.R., Rosselló J.A. Non-functional plastid ndh gene fragments are present in the nuclear genome of Norway spruce (Picea abies L. Karsch): insights from in silico analysis of nuclear and organellar genomes. *Mol Gen Genom.* 2016;291(2):935–941. DOI 10.1038/srep09040.
- Rico L., Ogaya R., Barbeta A., Penuelas J. Changes in DNA methylation fingerprint of Quercus ilex trees in response to experimental field drought simulating projected climate change. *Plant Biol.* 2014; 16(2):419–427. DOI 10.1111/plb.12049.
- Ritonga A.W., Chozin M.A., Syukur M., Maharijaya A., Sobir S. Genetic variability, heritability, correlation, and path analysis in tomato (Solanum lycopersicum) under shading condition. *Biodiversitas J Biol Diversity*. 2018;19(4):1527–1531. DOI 10.13057/biodiv/ d190445.
- Ruhlman, T.A., Chang, W.J., Chen, J.J., Huang, Y.T., Chan, M.T., Zhang, J., Liao D.C., Blazier J.C., Jin X., Shih M.C., Jansen R.K., Lin C.S. NDH expression marks major transitions in plant evolution and reveals coordinate intracellular gene loss. *BMC Plant Boil*. 2015;15(1):100. DOI 10.1186/s12870-015-0484-7.
- Sanderson M.J., Copetti D., Búrquez A., Bustamante E., Charboneau J.L., Eguiarte L.E., Kumar S., Lee H.O., Lee J., McMahon M., Steele K., Wing R., Yang T.J., Zwickl D., Wojciechowski M.F. Exceptional reduction of the plastid genome of saguaro cactus (*Carnegiea gigantea*): Loss of the ndh gene suite and inverted repeat. *Am J Bot.* 2015;102(7):1115–1127. DOI 10.3732/ajb.1500184.
- Shi Y., Yan X., Zhao P., Yin H., Zhao X., Xiao H., Li X., Chen G., Ma X.F. Transcriptomic analysis of a tertiary relict plant, extreme xerophyte Reaumuria soongorica to identify genes related to drought adaptation. *PLoS One*. 2013;8(5):e63993. DOI 10.1371/journal. pone.0063993.
- Shin H.W., Lee N.S. Understanding plastome evolution in Hemiparasitic Santalales: Complete chloroplast genomes of three species, Den-

drotrophe varians, Helixanthera parasitica, and Macrosolen cochinchinensis. *PloS One.* 2018;13(7):e0200293. DOI 10.1371/journal. pone.0200293.

- Silva S.R., Diaz Y.C., Penha H.A., Pinheiro D.G., Fernandes C.C., Miranda V.F., Michael T.P. Varani A.M. The chloroplast genome of Utricularia reniformis sheds light on the evolution of the ndh gene complex of terrestrial carnivorous plants from the Lentibulariaceae family. *PLoS One.* 2016;11(10):e0165176. DOI 10.1371/journal. pone.0165176.
- Silva S.R., Michael T.P., Meer E.J., Pinheiro D.G., Varani A.M., Miranda V.F. Comparative genomic analysis of Genlisea (corkscrew plants – Lentibulariaceae) chloroplast genomes reveals an increasing loss of the ndh genes. *PLoS One.* 201813(1):e0190321. DOI 10.1371/journal.pone.0190321.
- Smith C.M., Clement S.L. Molecular bases of plant resistance to arthropods. Ann Rev Entomol. 2012;57:309–328. DOI 10.1146/annurevento-120710-100642.
- Sulistyowati D., Chozin M.A., Syukur M., Melati M., Guntoro D. Selection of shade-tolerant tomato genotypes. *J Applied Horticulture*. 2016;18(2):154–159.
- von Kohn C., Kiełkowska A., Havey M.J. Sequencing and annotation of the chloroplast DNAs and identification of polymorphisms distinguishing normal male-fertile and male-sterile cytoplasms of onion. *Genome.* 2013;56(12):737–742. DOI 10.1139/gen-2013-0182.
- Wang P., Duan W., Takabayashi A., Endo T., Shikanai T., Ye J. Y., Mi H. Chloroplastic NAD (P) H dehydrogenase in tobacco leaves

functions in alleviation of oxidative damage caused by temperature stress. *Plant Physiol.* 2006;141(2):465–474. DOI 10.1104/pp.105. 070490.

- Wicke S., Schneeweiss G.M., Depamphilis C.W., Müller K.F., Quandt D. The evolution of the plastid chromosome in land plants: gene content, gene order, gene function. *Plant Mol Biol.* 2011; 76(3–5):273–297. DOI 10.1007/s11103-011-9762-4.
- Wickett N.J., Fan Y., Lewis P.O., Goffinet B. Distribution and evolution of pseudogenes, gene losses, and a gene rearrangement in the plastid genome of the nonphotosynthetic liverwort, Aneura mirabilis (Metzgeriales, Jungermanniopsida). J Mol Evol. 2008;67(1):111–122. DOI 10.1007/s00239-008-9133-1.
- Wu C.S., Lin C.P., Hsu C.Y., Wang R.J., Chaw S.M. Comparative chloroplast genomes of Pinaceae: insights into the mechanism of diversified genomic organizations. *Gen Biol Evol*. 2011;3:309–319. DOI 10.1093/gbe/evr026.
- Yamori W., Shikanai T., Makino A. Photosystem I cyclic electron flow via chloroplast NADH dehydrogenase-like complex performs a physiological role for photosynthesis at low light. *Sci Reports*. 2015; 5:13908.

Acknowledgements. This work was supported by the RFBR grant No. 18-04-01203.

Conflict of interest. The authors declare no conflict of interest.