Zootaxa 3904 (3): 387–402
www.mapress.com/zootaxa/

Article

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)

http://dx.doi.org/10.11646/zootaxa.3904.3.4
http://zoobank.org/urn:lsid:zoobank.org:pub:AD438287-768C-4223-9AF7-B3C8C2C3BE32

Steinernema balochiense n. sp. (Rhabditida: Steinernematidae) a new entomopathogenic nematode from Pakistan

SHAHINA FAYYAZ[1,3], TABASSUM ARA KHANUM[1], SHAUKAT ALI[2], GHULAM SARWAR SOLANGI[1], MEHREEN GULSHER[1]& SALMA JAVED[1]

[1]*National Nematological Research Centre, University of Karachi, Karachi-75270, Pakistan*
[2]*Engineering Research Center of Biological Control, Ministry of Education, College of Natural Resource and Environment, South China Agricultural University, Wushan Road, Guangzhou City, P.R. China, 510642*
[3]*Corresponding author. E-mail: shahinafayyaz@gmail.com*

Abstract

A new species of entomopathogenic nematode (EPN) named *Steinernema balochiense* **n. sp.** belonging to the family Steinernematidae was isolated from *Psidium guajava* L., rhizophere soil samples of Balochistan, Pakistan. This new species belongs to the *carpocapsae* group. The new species can be separated from other described species by morphological and morphometrics characteristics as well as DNA sequence polymorphisms. This new nematode species is morphologically characterized by features of infective juveniles (IJ) and males. For the IJ average body length was (455; 415–528) µm, distance from anterior end to excretory pore (35; 32–38) µm, pharynx length (90; 85–98) µm, tail length (44.3; 40–51) µm, D% and E% values (39; 36–44) and (80; 70–92), respectively. For male specimens, the diagnostic characters included total body length (1330; 1135–1632) µm, gubernaculum length (44.4; 40–47) µm, D% (43.8; 40–51) and ratio of GS (63.8; 53–75). Morphological diagnostic traits for new species include the presence of a funnel shaped gubernaculum at the proximal end. *S. balochiense* **n. sp.** differs from infective stage juveniles of closest species *S. nepalense* by having 6 ridges vs 7 ridges in the lateral field. Molecular phylogenetic trees based on sequence of ITS-rDNA, D2D3 regions and the mitochondrial 12S rRNA gene supports the description of this nematode isolate as a new species.

Key words: new species, morphology, molecular taxonomy, ITS-rDNA, D2D3 region, 12S rRNA gene

Introduction

Entomopathogenic nematodes (EPN) belonging to the families Steinernematidae Travassos, 1927 and Heterorhabditidae Poinar, 1975 have been proved as potential biological control agents against a wide range of insect pests (Grewal *et al*., 2013; Ulug *et al*., 2014). EPN have numerous advantages that quantify them as commercially valuable biocontrol agents. They are highly effective and often surpass the control results achieved with chemical compounds. In contrast to chemicals, which should not be displaced by water in the soil and have to decay within few days, EPN are mobile and persistent. They recycle inside the host causing long term sustainable effect on the pest population. Beneficial nematodes are more specific and are less threat to the environment than chemical insecticide (Ehlers & Peters, 1995). EPN are found under diverse ecological conditions including cultivated fields, forests, grasslands, deserts and ocean beaches (Hominick *et al*., 1996). Traditionally, species delimitation have been based on morphology and cross breeding experiments (Hominick *et al*., 1997), but recently numerous molecular approaches have been applied to identify and observe the evolutionary relationship between different species of EPN (Nguyen & Hunt, 2007). The current study describes a new species of EPN, *Steinernema balochiense* **n. sp.** PAK.B.*S*.45 (PAK = Pakistan; B = Balochistan, *S* = *Steinernema*) from Balochistan, Pakistan, based on morphometrics and molecular evidence which is compared and contrasted with the previously known species of the same genus.

Accepted by A. Malan: 20 Nov. 2014; published: 7 Jan. 2015

Steinernema balochiense **n. sp.** can be differentiated from *S. nepalense*, by shorter length of gubernaculum (44; 40–47) vs (56; 47–60) µm; shorter E% (200; 173–256) vs (297; 243–365); lower GS% (64; 53–75) vs (79; 70–93), proximal end of gubernaculum funnel shaped vs not funnel shaped and 23 vs 25 genital papillae.

Cross-hybridization. Progeny was not observed from cross breeding treatments. Progeny was produced in the shelf cross controls.

Molecular characteristics. *Steinernema balochiense* **n. sp.** is characterized genetically by sequences of the ITS (JX135547), D2D3 region (JX068821) of rDNA and 12SmtRNA gene (JN571100). The length of the ITS1 + 5.8S + ITS2 sequence is 842 bp; ITS1 = 202 bp, and ITS2 = 307 bp and its composition is A = 0.2530, C = 0.1706, G = 0.2195, T = 0.3569. The sequence of the D2D3 region of *S. balochiense* **n. sp.** is 1031 bp long and its base composition is: A = 0.2290, C = 0.2123, G = 0.2742, T = 0.2845. While 12S mtRNA is 515 bp and its base composition is: A = 0.2984, C = 0.1417, G = 0.1940, T = 0.3654. Sequence length and composition of other species are presented in Table 3 and pairwise taxa comparison is given in Table 4.

Phylogenetic relationships. The phylogenetic relationships between 57 *Steinernema* species, based on ITS rDNA polymorphism are presented in Fig. 4. (For M.P, tree length = 4089, CI = 0.3706, RI = 0.6919, RC = 0.2721, HI = 0.6294). In this consensus tree three species, *S. balochiense* **n. sp.** *S. nepalense* and *S. surkhetense* formed a monophyletic group with bootstrap support of 73%. The phylogenetic relationships between 47 *Steinernema* species, based on D2D3 sequences are presented in Fig. 5 (Tree length = 2403, CI = 0.4976, RI = 0.6846, RC = 0.3812, HI = 0.5024). In this consensus tree, four species, *S. balochiense* **n. sp.**, *S. websteri*, *S. anatoliense* and *S. nepalense* formed a monophyletic group with bootstrap support of 100%. These observations provided further evidence for the distinctness of this species. The phylogenetic relationships between 17 *Steinernema* species based on 12S rRNA gene sequences are presented in Fig. 6 (Tree length = 733, CI = 0.5329, RI = 0.5858, RC = 0.3988, HI = 0.4671). The new species is placed as a sister taxon of *S. scapterisci*. Affiliation of these species is supported by a bootstrap value of 52%.

Future possibility as biocontrol agent. This new species *S. balochiense* **n. sp.** shall be used as a biopesticide against insect pests in order to minimize impact of synthetic chemical pesticides. The symbiotic bacteria of *S. balochiense* **n. sp**. will also be identified.

Acknowledgement

The present work was conducted under the Project No.PSF/ILP/S-KU/Bio (047) entitled "Molecular Characterization, Mass Production and Formulation of Entomopathogenic Nematodes", supported by Pakistan Science Foundation. under 17th Protocol on Science and Technology Cooperation between Pakistan and China.

References

Anis, M., Shahina, F., Reid, A.P. & Rowe, J. (2002) *Steinernema asiaticum* n. sp. (Rhabditida: Steinernematidae) from Pakistan. *International Journal of Nematology*, 12, 220–231.

Baliadi, Y., Kondo, E. & Yoshiga, T. (2009) The continual forming and contribution of infective juveniles produced via endotokia matricida of entomopathogenic nematodes in the family of Steinernematidae and Heterorhabditidae. *Indonesian Journal of Agricultural Science*, 10, 26–33.
http://dx.doi.org/10.1303/aez.2004.61

Bedding, R.A. & Akhurst, R.J. (1975) A simple technique for the detection of insect parasitic rhabditid nematodes in soil. *Nematologica*, 21, 109–110.
http://dx.doi.org/10.1163/187529275x00419

Courtney, W.D., Pooley, D. & Miller, V.L. (1955) TAF, an improved fixative in nematode technique. *Plant Disease Reporter*, 39, 570–571.

Cutler, C.G. & Stock, S.P. (2003) *Steinernema websteri* sp. n. (Rhabditida: Steinernematidae), a new entomopathogenic nematode from China. *Nematologia Mediterranea*, 31, 215–224.

Ehlers, R.U. & Peters, A. (1995) Entomopathogenic nematodes in biological control: feasibility, perspectives and possible risk. *In*: Hokkanen, H.M.T. & Lynch, J.M. (Eds.), *Biological Control: Benefits and Risks*. Cambridge University Press, UK, pp. 119–136.
http://dx.doi.org/10.1017/cbo9780511661730.013

Doucet, M.M.A. de (1986) A new species of *Neoaplectana* Steiner, 1929 (Nematoda: Steinernematidae) from Cordoba,

Argentina. *Revue de Nematologie*, 9, 317–323.
Doucet, M.M.A. de & Doucet, M.E. (1990) *Steinernema ritteri* sp. n. (Nematoda: Steinernematidae) with a key to the species of the genus. *Nematologica*, 36, 257–265.
http://dx.doi.org/10.1163/002925990x00257
Grewal, P.S., Bai, X., Adams, B.J., Ciche, T.A., Clifton, S., Gaugler, R., Kim, K., Spieth, J., Sternberg, P.W. & Wilson, R.K. (2013) First complete genome sequence of an entomopathogenic nematode goes public. *Journal of Nematology*, 45, 291.
Hall, T.A. (1999) BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series*, 41, 95–98.
Hazir, S., Stock, S.P. & Keskin, N. (2003) A new entomopathogenic nematode *Steinernema anatoliense* sp. n. (Rhabditida: Steinernematidae) from Turkey. *Systematic Parasitology*, 55, 211–220.
Hominick, W.M., Briscoe, B.R., del Pino, F.G., Heng, J., Hunt, D.J., Kozodoy, E., Mracek, Z., Nguyen, K.B., Reid, A.P., Spiridonov, S., Stock, P., Sturhan, D., Waturu, C. & Yoshida, M. (1997) Biosystematics of entomopathogenic nematodes: currents status, protocols and definitions. *Journal of Helminthology*, 71, 271–298.
http://dx.doi.org/10.1017/s0022149x00016096
Hominick, W.M., Reid, A.P., Boham, A.P. & Briscoe, B.R. (1996) Entomopathogenic nematodes: biodiversity, geographical distribution and the convention on biological diversity. *Biological Science and Technology*, 6, 317–331.
http://dx.doi.org/10.1080/09583159631307
Joyce, S.A., Reid, A.P., Driver, F. & Curran, J. (1994) Application of polymerase chain reaction (PCR) methods to identification of entomopathogenic nematodes. *In*: Burnell, A.M., Ehlers, R.U. & Masson, J.P. (Eds.), *COST 812 Biotechnology: Genetics of Entomopathogenic Nematodes-Bacterium Complexes*, *Proceedings of Symposium & Workshop*. St. Patrick's College, Maynooth, Co., Kildare, Ireland. European Commission, DGXII, Luxembourg, pp. 178–187.
Kaya, H.K. & Stock, S.P. (1997) Techniques in insect nematology. *In*: Lacey, I. (Ed.), *Manual of techniques in insect pathology*. San Diego, CA, USA, Academic Press, pp. 313–314.
Khatri-Chhetri, H.B., Waeyenberge, L., Spiridonov, S., Manandhar, H.K. & Moens, M. (2011) Two new species of *Steinernema* Travassos, 1927 with short infective juveniles from Nepal. *Russian Journal of Nematology*, 19, 53–74.
López-Núñez, J.C., Plichta, K., Góngora-Botero, C.E. & Stock, S.P. (2008) A new entomopathogenic nematode, *Steinernema colombiense* sp. n. (Nematoda: Steinernematidae) from Colombia. *Nematology*, 10, 561–574.
Luc, P.V., Nguyen, K.B., Reid, A.P. & Spiridonov, S.E. (2000) *Steinernema tami* sp. n. (Rhabditida: Steinernematidae) from Cat Tien Forest, Vietnam. *Russian Journal of Nematology*, 8, 33–43.
Mamiya, Y. (1988) *Steinernema kushidai* sp. n. (Nematoda: Steinernematidae) associated with scarabaeid beetle larvae from Shizuoca, Japan. *Applied Entomology and Zoology*, 23, 313–320.
Nadler, S.A., Bolotin, E. & Stock, S.P. (2006) Phylogenetic relationship of *Steinernema* (Cephalobina, Steinernematidea) based on nuclear, mitochondrial and morphological data. *Systematic Parasitology*, 63, 159–179.
http://dx.doi.org/10.1007/s11230-005-9009-3
Nguyen, K.B. & Smart, G.C. Jr. (1990) *Steinernema scapterisci* sp. n. (Steinernematidae: Nematoda). *Journal of Nematology*, 22, 187–199.
Nguyen, K.B. & Hunt, D. (2007) *Entomopathogenic Nematodes: Systematics*, *Phylogeny and Bacterial Symbionts*. Brill, NV, Leiden, The Netherlands, 816 pp.
http://dx.doi.org/10.1163/ej.9789004152939.i-816
Nguyen, K.B., Malan, A.P. & Gozel, U. (2006) *Steinernema khoisanae* sp. n. (Rhabditida: Steinernematidae), a new entomopathogenic nematode from South Africa. *Nematology*, 8, 157–175.
http://dx.doi.org/10.1163/156854106777998728
Phan, K.L., Spiridonov, S.E., Subbotin, S.A. & Moens, M. (2006) Four new species of *Steinernema* Travassos, 1927 with short infective juveniles from Vietnam. *Russian Journal of Nematology*, 14, 11–29.
Poinar, G.O. (1975) Description and biology of new insect parasitic rhabditoid, *Heterorhabditis bacteriophora* gen. n., sp. n. (Rhabditida; Heterorhabditidae n. fam.). *Nematologica*, 21, 463–470.
http://dx.doi.org/10.1163/187529275x00239
Seinhorst, J.W. (1959) A rapid method for the transfer of nematodes from fixative to anhydrous glycerine. *Nematologica*, 4, 67–69.
http://dx.doi.org/10.1163/187529259x00381
Stock, S.P., Somsook, V. & Reid, A.P. (1998) *Steinernema siamkayai* sp. n. (Rhabditida: Steinernematidae), an entomopathogenic nematode from Thailand. *Systematic Parasitology*, 41, 105–113.
Swofford, D.L. (2001) PAUP: *Phylogenetic Analysis using Parsimony* (*and other methods*), *version 4*. Sunderland, MA, USA: Sinauer Associates (DVD). Available from: http://paup.csit.fsu.edu (accessed 3 September 2014)
Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F. & Higgins, D.G. (1997) The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acid Research*, 25, 4876–4882.
Travassos, L. (1927) Sobre O genero oxysomatium. *Boletim Biologico*, *Sao Paulo*, 5, 20–21.
Ulug, D., Hazir, S., Kaya, H.K. & Lewis, E. (2014) Natural enemies of natural enemies: the potential top-down impact of predators on entomopathogenic nematode populations. *Ecological Entomology*, 39, 462–469.
http://dx.doi.org/10.1111/een.12121

Weiser, J. (1955) *Neoaplectana carpocapsae* sp. n. (Anguillulata, Steinernematinae), novy cizopasnik housenek obalece jablecneho, *Carpocapsa pomonella* L. *Věstník Československé zoologické společnosti*, 19, 44–52.
White, G.F. (1927) A method for obtaining infective nematode larvae from cultures. *Science*, 66, 302–303. http://dx.doi.org/10.1126/science.66.1709.302-a
Wouts, W.M., Mracek, Z., Gerdin, S. & Bedding, R.A. (1982) *Neoaplectana* Steiner, 1929 a junior synonym of *Steinernema* Travassos, 1927 (Nematoda: Rhabditida). *Systematic Parasitology*, 4, 147–154. http://dx.doi.org/10.1007/bf00018998
Zuccon, A. & Zuccon, D. (2010) *MrEntv.2.1*. Program distributed by the authors. Available from: http: //www.mrent.org (accessed 3 September 2014)