# THE *XIPHINEMA AMERICANUM* GROUP. III. KEYS TO SPECIES IDENTIFICATION

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**Summary.** A polytomous and a dichotomous key for specific identification of 49 putative species of the *Xiphinema americanum* group are presented. Priority is given to quantitative characters to minimize dependency on subjective judgements. Among the characters selected odontostyle length, ratios *c* and *c'*, and V% appeared the most reliable for examining intra- and inter-population relationships.

Identification of species within the *Xiphinema americanum* group is of particular importance for phytosanitary regulation, but is problematic as a result of the general similarity of the morphology of the putative species. Consequently, EC legislation (EC Council Directive 2000/29/EC) lists non-European population of *X. americanum sensu lato* in Annex IAI in order to prevent the introduction of non-indigenous viruses to Europe. Recently, Lamberti *et al.* (2000) presented a series of regional polytomous identification keys, together with a polytomous key to the species occurring world-wide. These keys provided the first comprehensive attempt to resolve the problems with the identification of the *Xiphinema americanum* group of species. The keys were prepared within the framework of EC Contract N° SMT 1506, DGXII/C-5, MO 75 "*Xiphinema americanum* group virus vector nematodes: development of a diagnostic protocol". Subsequently, a workshop was held from 29th April to 1st May 2002 at the Central Science Laboratory (CSL), York, England, to test the robustness of the identification keys. Participants attending the workshop were provided with specimens belonging to several populations of putative *X. americanum* group species and requested to identify the nematodes using the identification keys that had been prepared by Lamberti *et al.* (2000). The results of this exercise identified that several improvements to the keys were necessary, and that several participants preferred working with a dichotomous key.

Here we present a revised polytomous key and include a dichotomous key accepting the suggestion of Luc and Baujard (2001) that dichotomous keys can be used to complement a polytomous key in which several species share the same code for one or more characters. The two *species inquirendae*, *X. neoamericanum* and *X. sharmai*, have been omitted from both keyhs. This is because of the poor quality of their original descriptions and the fact that neither species has been unequivocally identified after the publication of their original description; thus they are considered to have little relevance for phytosanitary regulation.

In both keys, priority is given to quantitative morphological characters to minimise subjective evaluation of qualitative characters. Lamberti *et al.* (2000) stated that odontostyle length, ratios *c* and *c'*, and V% appeared more reliable for examining intra- and inter-population relationships. When used as principal discriminant, relatively small groups of species were formed, within which demarcation of the individual species could be made using less robust characters such as body length, ratio *a*, and tail length, and also subjective characters such as lip region and tail shape.

For species authorities please see Lamberti *et al.*, 2000.

## Characters used in the polytomous key

**A:** **1** odontostyle length 70 µm and less;
**2** odontostyle length 71-80 µm;
**3** odontostyle length 81-90 µm;
**4** odontostyle length 91-100 µm;
**5** odontostyle length 101-120 µm;
**6** odontostyle length >120 µm;

**B:** **1** vulva (V%) 50% and less;
**2** vulva 51 to 54%;
**3** vulva 55 to 58%;
**4** vulva >58%;

**C:** **1** value of *c'* ratio 1.0 or less;
**2** value of *c'* ratio 1.1 – 1.4;
**3** value of *c'* ratio 1.5 – 1.8;
**4** value of *c'* ratio >1.8;

**D:** **1** value of *c* ratio <60;
**2** value of *c* ratio 60-80;
**3** value of *c* ratio >80;

**E: 1** body length <1.5 mm;
**2** body length 1.5-2.0 mm;
**3** body length >2.0 mm;

**G: 1** lip region continuous with body profile;
**2** lip region set off from body profile;

**H: 1** tail with pointed terminus;
**2** tail with rounded terminus;

**I: 1** tail length <27 µm;
**2** tail length 27-32 µm;
**3** tail length >32µm;

Among the species having 2 for code A, *X.citricolum* and *X. intermedium*, share all the other key characters. However, they can be separated on the basis of their tail shape, straight in *X. citricolum* and ventrally curved and concave in *X. intermedium* (a).

The following dichotomous key is based on the most robust diagnostic characters within the *X. americanum* group.

## Dichotomous key of *Xiphinema americanum* group species

| | | |
|---|---|---|
| **1.** | Odontostyle length >150 µm | *X. bacaniboia* |
| | Odontostyle length <150 µm | 2 |
| **2.** | Odontostyle length µm >120 µm | *X. silvaticum* |
| | Odontostyle length 120 µm and less | 3 |
| **3.** | Odontostyle length 101-119 µm | 4 |
| | Odontostyle length <101 µm | 12 |
| **4.** | Odontostyle length 101-106 µm | 5 |
| | Odontostyle length 107-119 µm | 8 |
| **5.** | Value of *c'* ratio >1.5 | 6 |
| | Value of *c'* ratio <1.5 | 7 |
| **6.** | Body length around 2.6 mm | *X. fortuitum* |
| | Body length around 2.2 mm | *X. madeirense* |
| **7.** | Tail length <30 µm | *X. brevicollum* |
| | Tail length >33 µm | *X. paramonovi* |
| **8.** | Value of *c'* ratio 0.5-1.0 | 9 |
| | Value of *c'* ratio >1.0 | 11 |
| **9.** | Vulva (V%) around 52% | *X. himalayense* |
| | Vulva (V%) around 55% | 10 |
| **10.** | Value of *c* ratio around 70 | *X. sheri* |
| | Value of *c* ratio around 90 | *X. pseudoguirani* |
| **11.** | Body length around 2 mm | *X. georgianum* |
| | Body length >2.5 mm | *X. longistilum* |
| **12.** | Odontostyle length 91-100 µm | 13 |
| | Odontostyle length 90 µm and less | 21 |
| **13.** | Vulva >55% | 14 |
| | Vulva 55% and less | 15 |
| **14.** | Value of *c* ratio around 70 | *X. incertum* |
| | Value of *c* ratio around 100 | *X. mesostilum* |
| **15.** | Value of *c'* ratio 1.0 or less | *X. taylori* |
| | Value of *c'* ratio >1.0 | 16 |
| **16.** | Value of *c* ratio <50 | 17 |
| | Value of *c* ratio >50 | 18 |
| **17.** | Value of *c'* ratio >1.5 | *X. neoelongatum* |
| | Value of *c'* ratio <1.5 | *X. franci* |
| **18.** | Vulva 53-54% | 19 |
| | Vulva 51-52% | 20 |
| **19.** | Value of *c* ratio around 50 | *X. parvum* |
| | Value of *c* ratio around 60 | *X. utahense* |
| **20.** | Rounded tail terminus | *X. luci* |
| | Pointed tail terminus | *X. floridae* |
| **21.** | Odontostyle length 81-90 µm | 22 |
| | Odontostyle length 80 µm and less | 39 |
| **22.** | Odontostyle length 86-90 µm | 23 |
| | Odontostyle length 80-85 µm | 32 |
| **23.** | Vulva >55% | *X. incertum* |
| | Vulva <55% | 24 |
| **24.** | Value of *a* ratio >50 | 25 |
| | Value of *a* ratio <50 | 26 |
| **25.** | Tail length around 30 µm | *X. californicum* |
| | Tail length around 33 µm | *X. bricolense* |
| **26.** | Value of *c* ratio <55 | 27 |
| | Value of *c* ratio >55 | 28 |
| **27.** | Value of *c'* ratio >1.5 | *X. citricolum* |
| | Value of *c'* ratio <1.5 | *X. franci* |
| **28.** | Rounded tail terminus | 29 |
| | Pointed tail terminus | 30 |
| **29.** | Tail length around 25 µm | *X. diffusum* |
| | Tail length around 30 µm | *X. incognitum* |
| **30.** | Lip region continuous with the rest of body | *X. inaequale* |
| | Lip region set off from the rest of body | 31 |
| **31.** | Odontostyle length 90 µm and more | *X. floridae* |
| | Odontostyle length <90 µm | *X. peruvianum* |
| **32.** | Vulva >56% | 33 |
| | Vulva <55% | 34 |
| **33.** | Vulva >56-57% | *X. pachtaicum* |
| | Vulva <57% | *X. pachydermum* |
| **34.** | Value of *a* ratio >50 | 35 |
| | Value of *a* ratio 50 or less | 36 |
| **35.** | Tail length around 40 µm | *X. pacificum* |
| | Tail length around 35 µm | *X. santos* |
| **36.** | Lip region continuous with the rest of body | 37 |
| | Lip region set off from the rest of body | 38 |
| **37.** | Value of *a* ratio around 40 | *X. rivesi* |
| | Value of *a* ratio >45 | *X. laevistriatum* |
| **38.** | Body length >1.5 mm | *X. oxycaudatum* |
| | Body length <1.5 mm | *X. tarjanense* |
| **39.** | Odontostyle length 72-80 µm | 40 |
| | Odontostyle length 70 µm and less | 49 |
| **40.** | Vulva <50% | *X. kosaigudense* |
| | Vulva 50% and more | 41 |
| **41.** | Vulva >55% | 42 |
| | Vulva <55% | 43 |
| **42.** | Value of *a* ratio around 60 | *X. pachtaicum* |
| | Value of *a* ratio >80 | *X. microstilum* |
| **43.** | Value of *c* ratio >50 | 44 |
| | Value of *c* ratio <50 | 46 |
| **44.** | Body length >2 mm | *X. occiduum* |
| | Body length 2 mm or less | 45 |
| **45.** | Value of *a* ratio >50 | *X. thornei* |
| | Value of *a* ratio <50 | *X. tenuicutis* |
| **46.** | Lip region continuous with the rest of body | *X. laevistriatum* |
| | Lip region set off from the rest of body | 47 |
| **47.** | Lip region frontally rounded | *X. americanum* |
| | Lip region frontally flattened | 48 |
| **48.** | Tail straight | *X. citricolum* |
| | Tail ventrally curved and concave | *X. intermedium* |
| **49.** | Vulva >55% | 50 |
| | Vulva <55% | 52 |
| **50.** | Value of *a* ratio around 60 | *X. opisthohysterum* |
| | Value of *a* ratio >70 | 51 |
| **51.** | Odontostyle length around 60 µm | *X. brevisicum* |
| | Odontostyle length around 70 µm | *X. duriense* |
| **52.** | Value of *c'* ratio <1.5 | *X. pakistanense* |
| | Value of *c'* ratio >1.5 | 53 |
| **53.** | Value of *c* ratio >55 | *X. simile* |
| | Value of *c* ratio <55 | 54 |
| **54.** | Lip region continuous with the rest of body | *X. minor* |
| | Lip region set off from the rest of body | 55 |
| **55.** | Body length >1.5 mm | *X. americanum* |
| | Body length <1.5 mm | *X. lambertii.* |

**Polytomous key of *Xiphinema americanum* group species.**

| Species | Identification code | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | A | B | C | D | E | F | G | H | I |
| *brevisicum* | 1 | 3 | 4 | 1 | 3 | 3 | 2 | 1 | 3 |
| *duriense* | 1 | 4 | 4 | 1 | 2 | 2 | 2 | 1 | 2 |
| *lambertii* | 1 | 2 | 4 | 1 | 1 | 1 | 2 | 1 | ? |
| *minor* | 1 | 2 | 3 | 1 | 2 | 1 | 1 | 1 | 2 |
| *opisthohysterum* | 1 | 3 | 4 | 1 | 2 | 1/2 | 2 | 1 | 2/3 |
| *pakistanense* | 1 | 2 | 2 | 1 | 2 | 1 | 2 | 1 | 2 |
| *simile* | 1 | 2/3 | 3 | 2 | 2/3 | 2 | 2 | 2 | 2/3 |
| *americanum* (a) | 2 | 1 | 3/4 | 1 | 2 | 1 | 2 | 1 | 3 |
| *citricolum* (a) | 2/3 | 2 | 3 | 1 | 2 | 1 | 2 | 1 | 3/2 |
| *intermedium* | 2 | 2 | 3 | 1 | 2 | 1 | 2 | 1 | 3/2 |
| *kosaigudense* | 2 | 1 | ? | 1 | 1 | 1 | 2 | 1 | ? |
| *laevistriatum* | 2/3 | 2 | 3 | 1 | 2 | 1 | 1 | 1 | 3/2 |
| *microstilum* | 2 | 3 | 3 | 2 | 3 | 3 | 2 | 1 | 1 |
| *occiduum* | 2 | 2 | 2 | 2 | 3 | 1 | 2 | 2 | 2 |
| *pachtaicum* | 2/3 | 3 | 3 | 2 | 2/3 | 2 | 2 | 1 | 2 |
| *tenuicutis* | 2 | 2 | 3 | 2 | 2 | 1 | 2 | 1 | 2 |
| *thornei* | 2 | 2 | 2 | 2 | 2/3 | 1 | 2 | 2 | 2 |
| *bricolense* | 3 | 2 | 3 | 1 | 2 | 1 | 2 | 1 | 3 |
| *californicum* | 3 | 2 | 3 | 2 | 2/3 | 1/2 | 2 | 1 | 2/3 |
| *diffusum* | 3 | 1 | 1 | 2 | 2 | 1 | 2 | 2 | 1 |
| *floridae* | 3/4 | 2 | 2 | 2 | 2 | 1 | 2 | 1 | 2/3 |
| *franci* | 3/4 | 2 | 2 | 1 | 1/2 | 1 | 2 | 1 | 2 |
| *inaequale* | 3 | 2 | 2 | 2 | 2/3 | 1 | 1 | 1 | 2 |
| *incertum* | 3/4 | 3 | 3 | 2 | 2 | 1 | 2 | 2 | 2 |
| *incognitum* | 3 | 2 | 2 | 2 | 2 | 1 | 2 | 2 | 2 |
| *oxycaudatum* | 3 | 2 | 3 | 1 | 2 | 1 | 2 | 1 | 3/2 |
| *pachydermum* | 3 | 4 | 3 | 2 | 3 | 2 | 2 | 1 | 2 |
| *pacificum* | 3 | 2 | 3 | 1 | 2 | 2 | 2 | 2 | 3 |
| *peruvianum* | 3/4 | 2 | 2 | 1 | 2 | 1 | 2 | 1 | 2 |
| *rivesi* | 3 | 2 | 2 | 1 | 2 | 1 | 1 | 2 | 3 |
| *santos* | 3 | 2 | 3 | 1 | 2 | 1 | 2 | 2 | 3 |
| *tarjanense* | 3 | 2 | 3 | 1 | 1 | 1 | 2 | 1 | 3 |
| *luci* | 4 | 2 | 2 | 2 | 2 | 1 | 1 | 2 | 2 |
| *mesostilum* | 4 | 3 | 3 | 3 | 3 | 3 | 2 | 1 | 1 |
| *neoelongatum* | 4 | 3 | 3 | 1 | 1 | 1 | 2 | 1 | ? |
| *parvum* | 4 | 2 | 2 | 2 | 2 | 1 | 2 | 2 | 1 |
| *taylori* | 4 | 1 | 1 | 3 | 3 | 1 | 2 | 2 | 1/2 |
| *utahense* | 4 | 2 | 2 | 2 | 3 | 2 | 2 | 2 | 3 |
| *brevicollum* | 5 | 2 | 1 | 2/3 | 2/3 | 1 | 2 | 2 | 1/2 |
| *fortuitum* | 5 | 2 | 4 | 2 | 3 | 3 | 2 | 1 | 3 |
| *georgianum* | 5 | 2 | 2 | 2 | 2 | 1 | 2 | 1 | 1/2 |
| *himalayense* | 5 | 2 | 1 | 3 | 3 | 1 | 1 | 2 | 2 |
| *longistilum* | 5 | 3 | 3 | 2 | 3 | 2 | 2 | 1 | 3 |
| *madeirense* | 5 | 3 | 4 | 1 | 3 | 2 | 2 | 1 | 3 |
| *paramanovi* | 5 | 2 | 2 | 1 | 3 | 1 | 2 | 2 | 3 |
| *pseudoguirani* | 5 | 2 | 1 | 3 | 2 | 1 | 2 | 2 | 1 |
| *sheri* | 5 | 3 | 1 | 2 | 2 | 1 | 2 | 2 | 1 |
| *bacaniboa* | 6 | 3 | 1 | 3 | 2 | 1 | 1 | 2 | 2 |
| *silvaticum* | 6 | 2 | 1 | 2 | 3 | 1 | 1 | 2 | 2 |

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## LITERATURE CITED

Lamberti F., Molinari S., Moens M. and Brown D.J.F., 2000. The *Xiphinema americanum*-group. I. Putative species, their geographical occurrence and distribution, and regional polytomous identification keys for the group. *Russian Journal of Nematology, 8*: 65-84.

Luc M. and Baujard P., 2001. On specific determination within the *Xiphinema americanum* group (Nematoda: Longidoridae). *Nematology, 3*: 727-728.