# Identification of Hirudo Species from Different Sources

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**Abstract** [**Objectives**] To identify the species of Hirudo from different sources. [**Methods**] In accordance with morphological, anatomical and molecular taxonomic characteristics, the identification conclusion was drawn. [**Results**] The living samples of Hirudo belong to 1 order, 2 families, 3 genera and 5 species, and the dry samples of medicinal leeches belong to 4 species. [**Conclusions**] The sources of medicinal leeches are complex, and there is a problem that Hirudo from different basic sources are mixed for medication.

**Key words** Medicinal leeches, Species, Identification

## 1 Introduction

Hirudo is one of the traditional Chinese medicines, and it is salty, bitter and neutral in nature. It has the functions of breaking blood, removing blood stasis and dredging meridians, and is mostly used in the treatment of thrombotic diseases in clinic[1]. The 2020 edition of *Chinese Pharmacopoeia* takes the dried whole of *Whitmania pigra* Whitman, *Hirudo nipponica* Whitman or *Whitmania acranulata* Whitman as authentic sources[2-3]. The leech, also known as *W. pigra* Whitman, is one of the main species of Hirudo in China[4]. However, in recent years, with the continuous development of ready-for-use traditional Chinese medicine containing leeches, the demand for Hirudo has increased. Many species of Hirudo have been used as local medicinal materials in some areas or folk in China, and there are some problems such as mixture of similar species for medication and shoddy species for quality species. The traditional identification method of medicinal leeches is mainly through traits and thin layer identification, but when the medicinal materials are processed and crushed, the traits disappear, so the traditional identification method can not meet the actual work needs. In order to identify Hirudo species accurately, this study starts with morphology, anatomy and molecular biology, and accurately identifies the source of Hirudo, which lays a solid foundation for reducing the adulteration of leech Chinese medicines in the future, ensuring the authentic source of Chinese medicines and quality control.

## 2 Materials and methods

**2.1 Sample information** A total of 9 samples were used in this study, including 5 living samples and 4 dry samples (Table 1).

**2.2 Experimental instruments** Electronic balance (Shanghai Fangrui Company), ultra-clean workbench (Suzhou Jinghua Company), high-speed refrigeration centrifuge (Xiangyi Company), NanoDrop One ultra-micro spectrophotometer (Thermo Fisher Company), T100 PCR instrument (BIO RAD Company), gel electrophoresis system (Beijing Liuyi Company), gel imager (BIO RAD Company), body microscope (Olympus, Japan).

**Table 1 Medicinal leech sample information**

| No. | Sample status | Name | Origin |
|---|---|---|---|
| 1 | Living | *Whitmania pigra* Whitman | Weishan, Shandong Province |
| 2 | Living | *Hirudo tianjinensis* Liu | Yutai, Shandong Province |
| 3 | Living | *Poecilobdella manillensis* | Kunming, Yunnan Province |
| 4 | Living | *Hirudo nipponia* Whitman | Yutai, Shandong Province |
| 5 | Living | *Whitmania laevis* | Yutai, Shandong Province |
| 6 | Dry | *Eisenia andrei* | Daqing, Northeast China |
| 7 | Dry | *Barbronia weberi* | Daqing, Northeast China |
| 8 | Dry | – | Daqing, Northeast China |
| 9 | Dry | *Barbronia weberi* | Daqing, Northeast China |

**2.3 Experimental reagents** Broad-spectrum genomic DNA small quantity purification kit (Takara Company), Premix Taq reagent (Takara Company), agarose (Biowest Company), 6 × Loading Buffer (Takara Company), 5 × TBE buffer (Solarbio), GelRed nucleic acid dye (Biotium Company), DL1000 DNA marker (Takara Company).

**2.4 Morphological identification** 3 – 5 individuals were randomly selected from each sample, anesthetized, killed and fixed with 10% – 15% ethanol. The shape and structure of medicinal leeches were observed and described under a postural microscope, and typical individuals were selected for photographing. In addition, 2 – 3 fixed samples were dissected and observed under the postural microscope, their reproductive system pattern maps were drawn, and species morphological identification was carried out in accordance with relevant literature.

**2.5 DNA extraction and sequencing** 20 – 60 mg of samples were selected to extract genomic DNA according to the instructions of the broad-spectrum genomic DNA small quantity purification kit. The mitochondrial Cytochrome c oxidase subunit I (COI) gene fragment was amplified and sequenced by PCR with forward primer LCOI490: 5'-GGTCAACAAATCATAAAGATATTGG-3' and reverse primer HCO 2198: 5'-TAAACTTCAGGGTGACCAAAAAATCA-3', and the sequence of the mitochondrial COI gene fragment was obtained. Blast comparison was carried out in

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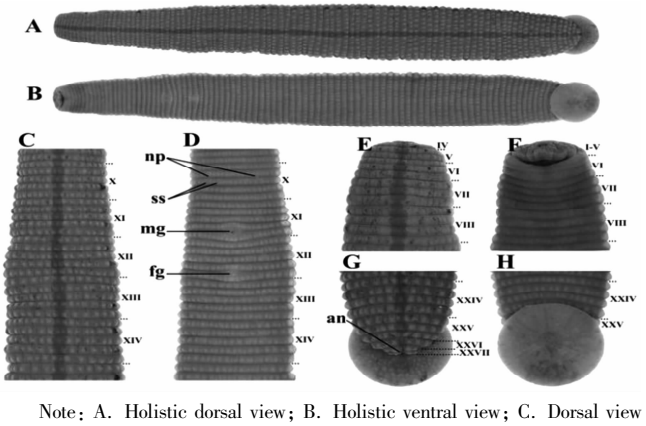
NCBI database, and the identified species were determined with reference to Query coverage and Percent identity, and the species molecular phylogenetic tree was constructed.

## 3 Identification results

**3.1 Living samples of Hirudo** According to the characteristics of morphology, anatomy and molecular taxonomy, the identification conclusion was drawn. The living samples of Hirudo collected this time belong to 1 order, 2 families, 3 genera and 5 species respectively. The scientific name, taxonomic status, identifying characteristics and molecular taxonomy results of the species are as follows:

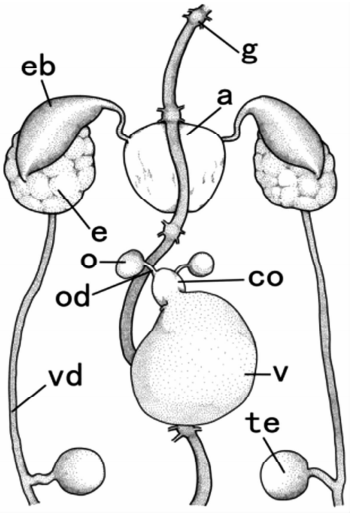
**3.1.1** *Poecilobdella manillensis* (Lesson, 1842). (i) Taxonomic status. *Poecilobdella*, Hirudinidae, Arhynchobdellida, Hirudinea. (ii) Identification characteristics. The body length was 95 – 118 mm, it could be extended to 215 mm, and the maximum body width was 20 – 24 mm; the tail cupule was 5 – 8 mm in diameter, which was obviously smaller than the body width; the body shape was long and narrow and flat, with olive green on the back, and the mastoid sensory organs were very obvious at rest; there was a blue-gray continuous or discontinuous longitudinal stripe in the middle of the back; the ventral surface was yellowish brown without spots; there was a thick yellow stripe on both sides of the body; there were 5 pairs of eyes, located in the $2^{nd}$, $3^{rd}$, $4^{th}$, $6^{th}$ and $9^{th}$ rings; three jaw slices were very obvious, with many mastoids on both sides; the crop had 10 pairs of lateral caeca, located between node IX and XVIII, one pair for each node; the male reproductive foramen was located at the posterior edge of $b_5/b_6$ in node XI, and the female reproductive foramen was located at the anterior edge of $b_5/b_6$ in node XII, with 5 rings between the two reproductive foramens; the ejaculatory duct was spindle-shaped; there were 11 pairs of testes.

The above morphological features were consistent with those of *P. manillensis* (Fig. 1 – 2).




Note: A. Holistic dorsal view; B. Holistic ventral view; C. Dorsal view of node X-XIV; D. Ventral view of node X-XIV; E. Dorsal view of node I-VIII; F. Ventral view of node I-VIII; G. Dorsal view of node XXIV-XXVII and tail cupule; H. Ventral view of node XXIV-XXVII and tail cupule. an. Anus; fg. Female genital pore; mg. Male genital pore; np. Nephridiopore; ss. Segmental receptors.

**Fig. 1 External morphology of *Poecilobdella manillensis***




Note: a. atrium; ag. albumen gland; co. common oviduct; e. seminal vesicle; eb. ejaculation ball; g. ganglia; o. ovary; p. prostate; ps. penile sac; te. testis; v. vagina; vd. vas deferens.

**Fig. 2 Reproductive system pattern diagram of *Poecilobdella manillensis***

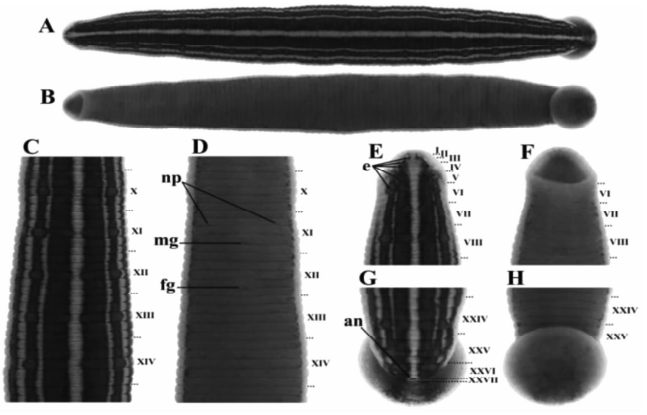
(iii) Molecular classification. The length of the mitochondrial COI gene fragment was 658 bp and the sequence was as follows:

AACTCTATATTTGATTCTAGGGGCTTGGGCAGCTATATTAGGC
TCCTCTATAAGAACTATTATTCGAATTGAGTTATCTCAACCAG
GTAGGTTTCTTGGGGATGATCAACTTTATAATTCTTTAATTACT
GCACATGGACTTATTATAATTTTTTTTATAGTAATACCTATTTT
AATCGGTGGGTTTGGTAATTGACTTTTACCGTTAATAATTGGT
GCCCCAGATATGGCTTTTCCACGATTAAATAATTTTAGGTTTT
GATTATTACCACCTTCATTAACTATATTAGTAAGATCATCAAT
AATTGAATCCGGTGTTGGTACAGGATGGACTATTTATCCACCA
TTAGCTGATAGAGTTTCTCACTCAGGACCTTGTGTAGATATAG
CTATCTTTTCATTGCATATAGCTGGTGCATCATCTATTTTAGGT
TCTTTAAATTTTATTTCTACTATTATTAATATGCGAACTAATGG
TATAAGTAATGAACGAGTTCCATTATTTGTTTGATCTGTTGTA
ATTACTACTATCTTATTATTACTTTCATTACCTGTATTAGCAGC
AGCTATTACAATGTTATTAACTGATCGTAATTTAAATACTTCA
TTTTTTGATCCAATGGGTGGTGGAGATCCAGTATTATTTCAAC
ACTTATTT

This sequence had high similarity with the corresponding sequence of *P. manillensis* in NCBI database (GeneBank accession number: MN882682).

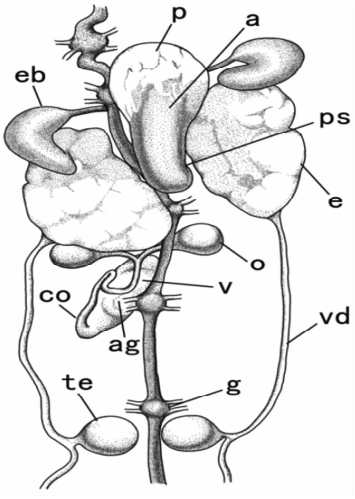
**3.1.2** *Hirudo tianjinensis* Liu, 2022. (i) Taxonomic status. *Hirudo*, Hirudinidae, Arhynchobdellida, Hirudinea. (ii) Identification characteristics. The body length was 32 – 38 mm (70 mm in cultured individuals), and the maximum width was 3.5 – 4.1 mm; the front cupule was 1.7 – 1.9 mm in diameter, the tail cupule was 3.4 – 3.8 mm in diameter, and the diameter of the tail cupule was slightly smaller than the maximum width. The body was long and narrow and flat, with olive green on the back. There were 5 continuous yellow longitudinal stripes, and the middle one was the widest, running through the whole back of the body. 5 dorsal longitudinal stripes and 2 light yellow side stripes divided the back into six longitudinal regions, with two wider regions in the middle and four narrower regions on both sides. There were six

sensory organs in a2 ring in the middle of node VIII-XXV. The ventral surface was gray-green, with a small number of irregular color spots on both sides and no sensory organs. The tail cupule was reddish brown, with darker back and lighter venter. There were 5 pairs of eyes, located in the 2nd, 3rd, 4th, 6th and 9th rings, and the last pair of eyes were very small and sometimes difficult to observe. The male genital pore was located on the $b_5/b_6$ ring groove of node XI, and the female genital pore was located on the $b_5/b_6$ ring groove of node XII, with 5 rings between the two genital pores. The anus was in the back between the last two rings. The prostate was white and conspicuous, covering the front half of the atrium; there were 11 pairs of testes. The above morphological features were consistent with those of *H. tianjinensis* Liu (Fig. 3 – 4).




Note: A. Holistic dorsal view; B. Holistic ventral view; C. Dorsal view of node X-XIV; D. Ventral view of node X-XIV; E. Dorsal view of node I-VIII; F. Ventral view of node I-VIII; G. Dorsal view of node XXIV-XXVII and tail cupule; H. Ventral view of node XXIV-XXVII and tail cupule. an. Anus; e. Eyes; fg. Female genital pore; mg. Male genital pore; np. Nephridiopore.

**Fig. 3 External morphology of *Hirudo tianjinensis* Liu**




Note: a. atrium; ag. albumen gland; co. common oviduct; e. seminal vesicle; eb. ejaculation ball; g. ganglia; o. ovary; p. prostate; ps. penile sac; te. testis; v. vagina; vd. vas deferens.

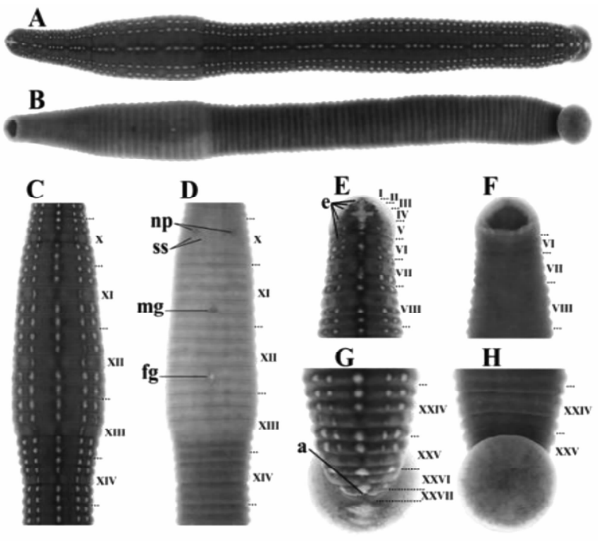
**Fig. 4 Reproductive system pattern diagram of *Hirudo tianjinensis* Liu**

(iii) Molecular classification. The length of the mitochondrial COI gene fragment was 658 bp and the sequence was as follows:
AACACTATATTTTATTTTGGGTGCTTGATCATCTATACTAGGT
TCTTCTATAAGATCCATTATTCGTATTGAGTTAGCTCAACCAG
GTAGATTTCTAGGGGATGATCAATTATATAATTCATTAGTAAC
TGCTCATGGGTTAGTTATAATTTTTTTTATAGTAATACCTATTT
TGATTGGTGGGTTTGGAAATTGATTATTGCCATTAATAGTTGG
AGCAGTTGATATATCATTTCCACGTCTTAATAATCTAAGCTTT
TGATTATTACCACCATCTATAATTATACTACTAAGTTCCTCAA
TAATTGAAGGTGGTGTAGGAGCTGGATGAACATTATATCCGC
CATTATCAGATTCAATATCACATTCTGGTCCGTCAGTAGATAT
AGCAATTTTTTCATTACATATAGCCGGGGCATCATCTATTTTG
GGATCTTTAAACTTTATTTCTACAATTATTAATATACGAACTA
ATGGAATAAGAGTTGAACGTATTCCATTATTTGTATGGTCAGT
AATTATTACTACTATTCTTCTACTTTTATCATTACCTGTTTTAGC
TGCAGCAATTACTATATTATTAACAGATCGAAATTTAAATACC
TCTTTTTTTGATCCTATAGGAGGGGGAGACCCAATTTTGTTTCA
ACACTTATTT

This sequence had high similarity with the corresponding sequence of *H. tianjinensis* Liu in NCBI database (GeneBank accession number: MZ820659).

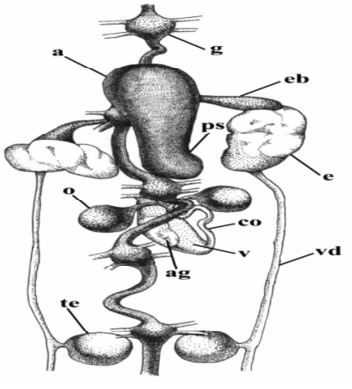
**3.1.3** *Hirudo nipponia* Whitman, 1886. (i) Taxonomic status. *Hirudo*, Hirudinidae, Arhynchobdellida, Hirudinea. (ii) Identification characteristics. The body length was 30 – 61 mm, and the maximum body width was 4.0 – 8.5 mm; the tail cupule was 3.5 – 5.5 mm, which was smaller than the body width; the body shape was long and narrow, slightly cylindrical. The back was olive green, with 5 discontinuous yellow longitudinal stripes, and the middle one was the widest. The 5 longitudinal stripes were usually composed of nearly square yellow-white patches on four consecutive rings, separated by a smaller patch (or a dark area) on one ring, so the longitudinal stripes appeared to be composed of rod-shaped stripes broken into small sections. The ventral surface was gray or yellowish brown without markings. 5 pairs of eyes were located in the 2nd, 3rd, 4th, 6th and 9th rings. The male genital pore was located on the $b_5/b_6$ ring groove of node XI, and the female genital pore was located on the $b_5/b_6$ ring groove of node XII. There were five rings between the two genital pores. The anus was located on the back between the last two rings. The prostate was inconspicuous, yarn-shaped, or even invisible; there were 11 pairs of testes. The above morphological features were consistent with those of *H. nipponia* Whitman (Fig. 5 – 6).

(iii) Molecular classification. The length of the mitochondrial COI gene fragment was 658 bp and the sequence was as follows:
TACATTATACTTTATTCTAGGAGCATGAGCATCCATGCTAGG
ATCTTCAATAAGATCCATTATTCGAATTGAGCTATCACAGCC
TGGGAGATTCCTAGGAGATGATCAATTATATAACTCACTAGT
AACTGCTCATGGGTTAGTTATAATTTTCTTTATGGTAATACCA
ATTCTGATTGGTGGCTTTGGTAATTGACTCCTTCCATTAATAG
TTGGAGCTGTCGATATATCCTTTCCACGTCTAAATAACCTAA
GGTTTTGGCTATTACCGCCCTCAATAATTATATTATTAAGTTC
ATCAATAATTGAAGGGGGGGTTGGAGCAGGCTGAACCCTAT
ATCCTCCCCTATCCGACTCAGTATCCCACTCAGGCCCATCAG
TAGATATAGCAATCTTCTCACTACATATAGCTGGTGCCTCCTC
TATCTTAGGCTCATTAAATTTTATTTCGACTATTATTAATATA




Note: A. Holistic dorsal view; B. Holistic ventral view; C. Dorsal view of node X-XIV; D. Ventral view of node X-XIV; E. Dorsal view of node I-VIII; F. Ventral view of node I-VIII; G. Dorsal view of node XXIV-XXVII and tail cupule; H. Ventral view of node XXIV-XXVII and tail cupule. an. Anus; e. Eyes; fg. Female genital pore; mg. Male genital pore; np. Nephridiopore; ss. Segmental receptors.

**Fig. 5 External morphology of *Hirudo nipponia* Whitman**




Note: a. Atrium; ag. Albumen gland; co. Common oviduct; e. Seminal vesicle; eb. Ejaculation ball; g. Ganglia; o. Ovary; p. Prostate; ps. Penile sac; te. Testis; v. Vagina; vd. Vas deferens.

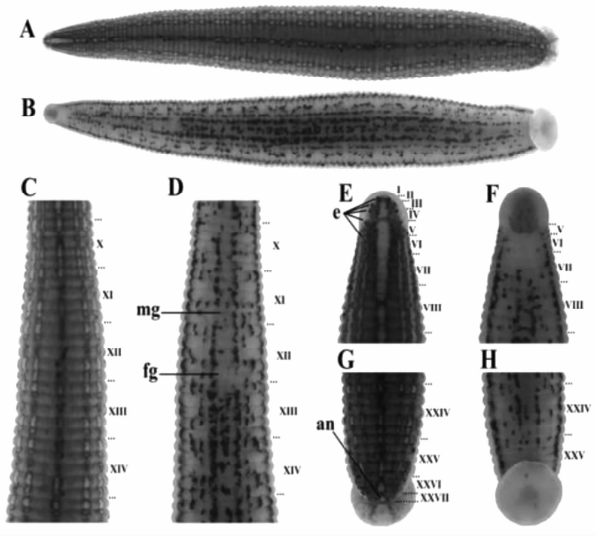
**Fig. 6 Reproductive system model diagram of *Hirudo nipponia* Whitman**

CGAACTAAGGGTATAAGAGCTGAACGTGTCCCATTATTTGTT
TGATCTGTAATTATTACTACTATTTTACTACTATTATCATTACC
AGTTCTAGCAGCAGCTATTACAATACTTTTAACTGATCGAAA
CCTAAATACTACATTCTTTGACCCTATAGGTGGTGGAGACCC
CATTTTATTTCAACACTTATTT

This sequence had high similarity with the corresponding sequence of *H. nipponia* Whitman in NCBI database (GeneBank accession number: MZ820662).

**3.1.4** *Whitmania pigra* Whitman (Whitman, 1884). (i) Taxonomic status. *Whitmania*, Haemopidae, Arhynchobdellida, Hirudinea. (ii) Identification characteristics. The body length was 60 – 130 mm, it could be extended to 180 mm, and the maximum body width was 18 – 24 mm; the tail cupule was 5 – 8 mm in diameter, which was obviously smaller than the body width; the body was slightly spindle-shaped and the back was dark green; there were five discontinuous longitudinal stripes in the middle of the back, one of which was darker and thicker; the ventral surface was light yellowish brown, with slightly longitudinal black-brown spots; there were 4 rings on the back of node VII and only 3 rings on the ventral surface; there were 5 pairs of eyes, located in the 2$^{nd}$, 3$^{rd}$, 4$^{th}$, 6$^{th}$ and 9$^{th}$ rings; the front cupule was small and the mouth was located inside the trailing edge; the crop had 11 contralateral caeca, located between node XIV and XXII; the male genital pore was located on the $b_5/b_6$ ring groove of node XI, and the female genital pore was located on the $b_5/b_6$ ring groove of node XII, with five rings between the two genital pores; there were 10 – 11 pairs of testes.

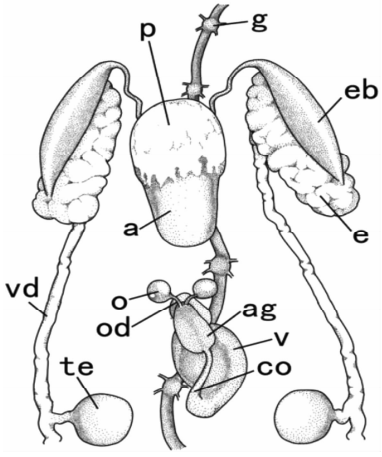
The above morphological features were consistent with the morphological features of *W. pigra* Whitman (Fig. 7 – 8).




Note: A. Holistic dorsal view; B. Holistic ventral view; C. Dorsal view of node X-XIV; D. Ventral view of node X-XIV; E. Dorsal view of node I-VIII; F. Ventral view of node I-VIII; G. Dorsal view of node XXIV-XXVII and tail cupule; H. Ventral view of node XXIV-XXVII and tail cupule. an. Anus; e. Eyes; fg. Female genital pore; mg. Male genital pore.

**Fig. 7 External morphology of *Whitmania pigra* Whitman**

(iii) Molecular classification. The length of the mitochondrial COI gene fragment was 658 bp and the sequence was as follows:
TACTTTATACTTTATTTTAGGAACGTGATCAGCTATGTTAGGC
TCTTCTATAAGATCAATTATTCGAATTGAATTAGCACAGCCAG
GAAGATTCCTTGGAGACGACCAATTGTATAATTCATTAGTAAC
GGCTCATGGGTTGGTTATAATCTTCTTTATAGTTATACCAATTC
TAATTGGTGGGTTTGGTAATTGACTCCTACCATTAATGGTAGG
GGCCGTAGATATATCGTTTCCTCGTCTGAATAATTTAAGATTT
TGGTTACTACCCCCTTCAATAATCATATTGCTTAGGTCATCCT




Note: a. Atrium; ag. Albumen gland; co. Common oviduct; e. Seminal vesicle; eb. Ejaculation ball; g. Ganglia; o. Ovary; p. Prostate; ps. Penile sac; te. Testis; v. Vagina; vd. Vas deferens.

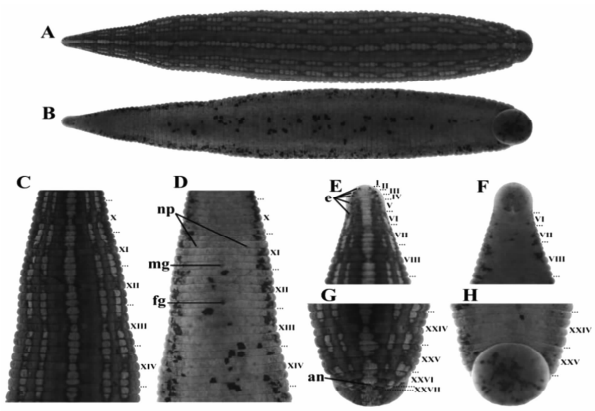
**Fig. 8 Reproductive system model diagram of *Whitmania pigra* Whitman**

TAATTGAGGGTGGTGTAGGTGCAGGGTGAACCCTTTATCCTC
CACTATCAGACTCCGTATCTCATTCAGGCCCATCCGTTGACA
TAGCCATCTTCTCATTACATATAGCTGGTGCCTCATCTATTTTA
GGGTCATTAAATTTTATTTCGACTATTATAAATATACGAACTA
AAGGAATAACAACTGAACGAGTACCATTATTTGTTTGGTCAGT
TGTTATTACTACTATTTTATTATTGTTATCATTACCAGTTTTAG
CAGCAGCTATTACAATATTACTTACAGATCGAAATTTAAATAC
TACTTTCTTTGACCCTATAGGAGGGGGGGATCCTATTTTGTTT
CAACATTTATTT

This sequence was similar to the corresponding sequence of *Whitmania pigra* Whitman in NCBI database (GeneBank accession number: MN729556).

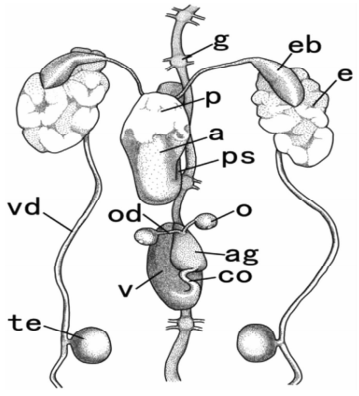
**3.1.5** *Whitmania laevis* (Baird, 1869). (i) Taxonomic status. *Whitmania*, Haemopidae, Arhynchobdellida, Hirudinea. (ii) Identification characteristics. The body length was 32 – 80 mm, and the maximum width was 5 – 12 mm; the tail cupule was 2 – 4 mm, which was obviously smaller than the body width; the body shape was long and narrow and flat, with olive green, light green and brown on the back, the color changed a lot, and the mastoid sensory organs were not obvious at rest; there were 5 discontinuous yellow longitudinal stripes in the middle of the back; both sides of the ventral surface were dark green, and the middle part was brownish yellow or brown, with irregular scattered black spots; there were 5 pairs of eyes, located in the $2^{nd}$, $3^{rd}$, $4^{th}$, $6^{th}$ and $9^{th}$ rings; the crop had 11 contralateral caeca; there were 4 rings on the dorsal and the ventral side of node VII; the male reproductive pore was on the $b_6$ ring of node XI, and the female reproductive pore was on the $b_6$ ring of node XII; there were 11 pairs of testes, globose or ovoid. The above morphological features were consistent with the morphological features of *W. laevis* (Fig. 9 – 10).

(iii) Molecular classification. DNA was extracted from the sample for many times, but the ideal sequence of mitochondrial COI fragment was not obtained.




Note: A. Holistic dorsal view; B. Holistic ventral view; C. Dorsal view of node X-XIV; D. Ventral view of node X-XIV; E. Dorsal view of node I-VIII; F. Ventral view of node I-VIII; G. Dorsal view of node XXIV-XXVII and tail cupule; H. Ventral view of node XXIV-XXVII and tail cupule. an. Anus; e. Eyes; fg. Female genital pore; mg. Male genital pore; np. Nephridiopore.

**Fig. 9 External morphology of *Whitmania laevis***




Note: a. Atrium; ag. Albumen gland; co. Common oviduct; e. Seminal vesicle; eb. Ejaculation ball; g. Ganglia; o. Ovary; p. Prostate; ps. Penile sac; te. Testis; v. Vagina; vd. Vas deferens.

**Fig. 10 Reproductive system model diagram of *Whitmania laevis***

## Key to the Above Five Species of Medicinal Leeches

1. The male genital pore was located on the b6 ring of node XI, and the female genital pore was located on the b6 ring of node XII, and the two pores were separated by 4 rings; there were 4 rings on the dorsal and ventral side of node VII ····· *W. laevis*
   The male genital pore was located on the $b_5/b_6$ ring groove of node XI, the female genital pore was located on the $b_5/b_6$ ring groove of node XII, and the two pores were separated by 5 rings; there were 3 rings on the dorsal and ventral side of node VII or 4 rings on the dorsal side and only 3 rings on the ventral side ····· 2
2. There were 4 rings on the dorsal side of node VII, and only 3 rings on the ventral side ····· *W. pigra* Whitman
   There were 3 rings on the dorsal and ventral side of node VII ····· 3
3. Individuals were large, and the mature individuals were more than 10 cm long; there was an orange-yellow or reddish-brown longitudinal band on each side of the body ····· ····· *P. manillensis*

Individuals were small, and the length of mature individuals was 5 – 7 cm; the longitudinal stripes on both sides of the body were lighter in color ····· 4

4. The yellow longitudinal stripes on the back were continuous and thick, and the middle one was the most prominent; the prostate gland was obvious ····· *H. tianjinensis* Liu
The yellow longitudinal stripes on the back were discontinuous, thin and spotted, and the longitudinal stripes where a2 ring was located were dark or missing; the prostate gland was not obvious ····· *H. nipponia* Whitman

**3.2 Dry samples of medicinal leeches** Molecular taxonomy of 4 dry samples of medicinal leeches was studied. DNA was successfully extracted from 4 samples and COI fragment sequences were obtained. The sequence alignment results are shown in Table 2.

**Table 2 Sequence alignment results of dry samples of medicinal leeches**

| No. | Name | Refer to Genbank ID | Identification results |
|---|---|---|---|
| 1 | *Eisenia andrei* | LC006116 | *Eisenia andrei* |
| 2 | *Barbronia weberi* | KU553102 | *Barbronia weberi* |
| 3 | Northeast *Hirudo* | MF358688 | *Erpobdella* (morphological identification is needed for species in accordance with living samples) |
| 5 | Unknown | KU553102 | Barbronia weberi |

## 3 Conclusion

There are many species of Hirudo circulating in the market at present, such as "Northeast *Hirudo*", "*E. andrei*", "*B. weberi*" and "*W. laevis*". In addition, some wild counterfeits including *Erpobdella*, *Hameadipsa* and non-*Hirudo* species are circulated and used as Hirudo in the market, and there is a problem that Hirudoof different origins are mixed with each other for medication. Moreover, these related species are difficult to distinguish and identify only by the current standards, which affects the quality and safety of genuine medicinal materials.

The current *Pharmacopoeia* standard has only a morphological description for the species control of Hirudo. When the medicinal materials are presented in dry state or processed in powder, they can hardly be identified by naked eyes. At the same time, it is found in practice that TLC identification method can not be used to distinguish genuine Hirudo from some counterfeit ones. To a certain extent, the lack of quality control indicators for qualitative identification and testing of this kind of standard Chinese herbal medicines brings difficulties to drug control, and makes the production enterprises or inspection departments unable to identify the species of Hirudo used, which may lead to the use of uncontrolled medicinal materials from unknown sources for drug production, and even affect the safety of clinical medication. Therefore, the next step will be to study and design primer pairs based on amplification of small fragments of genes, in order to quickly and accurately identify the sources of Hirudo in medicinal materials and traditional Chinese medicine prescriptions, and provide a reference for molecular biological identification of Hirudo.

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