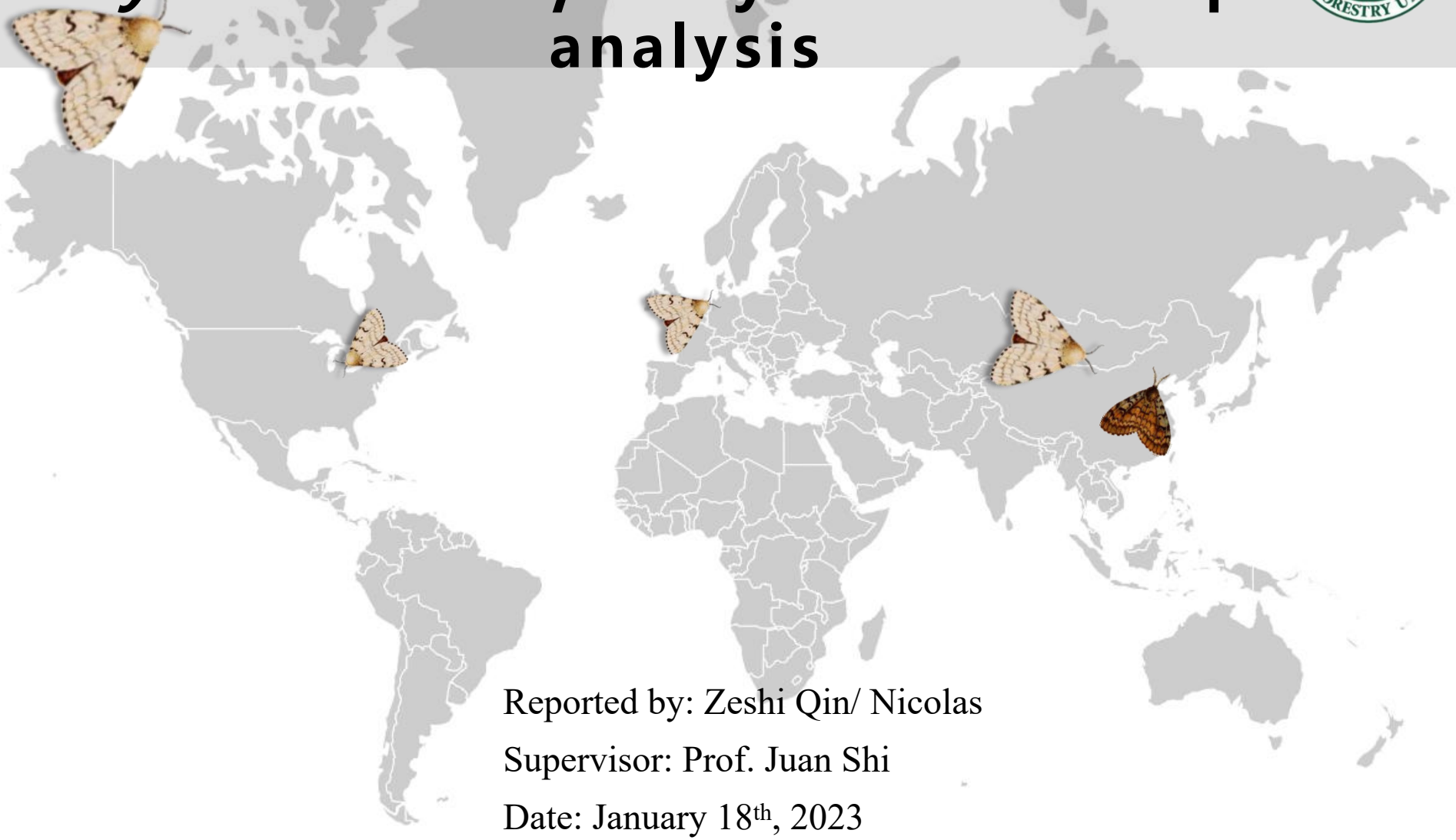
# Tracing the geographical origin of *Lymantria dispar* by stable isotope analysis



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Date: January 18th, 2023

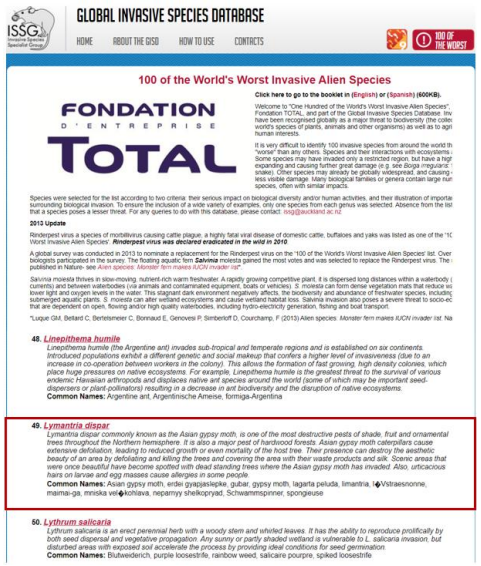
# 1 Research background

## 1.1 Object

Male ♂

Female ♀

- *Lymantria dispar* (L.) (Lepidoptera: Erebidae) (Boukouvala *et al*., 2022), an international quarantine pest, whose larval stage can feed on more than 500 species trees (Beurs *et al*., 2008; Kostić *et al*., 2008; Cao *et al*., 2015).
- The International Union for Conservation of Nature (IUCN) has listed *L.dispar* as one of the 100 worst invaders globally (Lowe *et al*., 2000).
- In 2016, more than 1 million acres of land were destroyed by spongy moths in the 20 states surveyed (USDA, 2018).



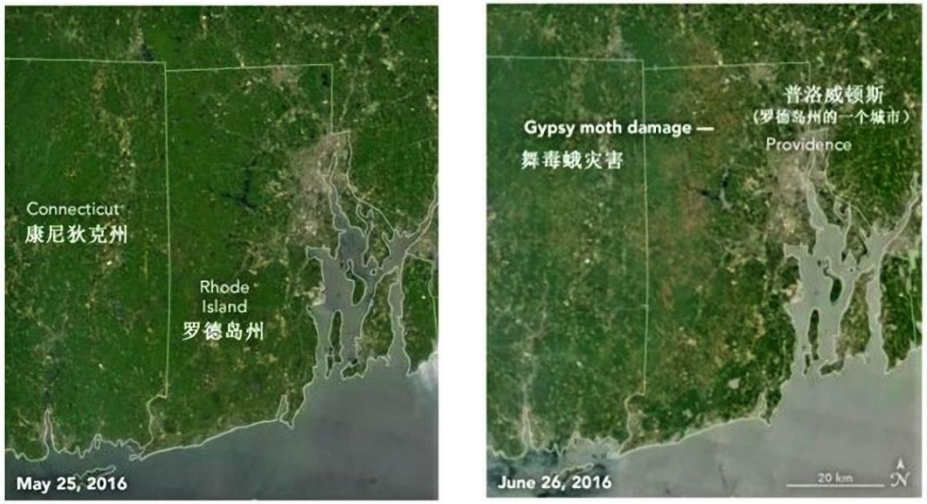
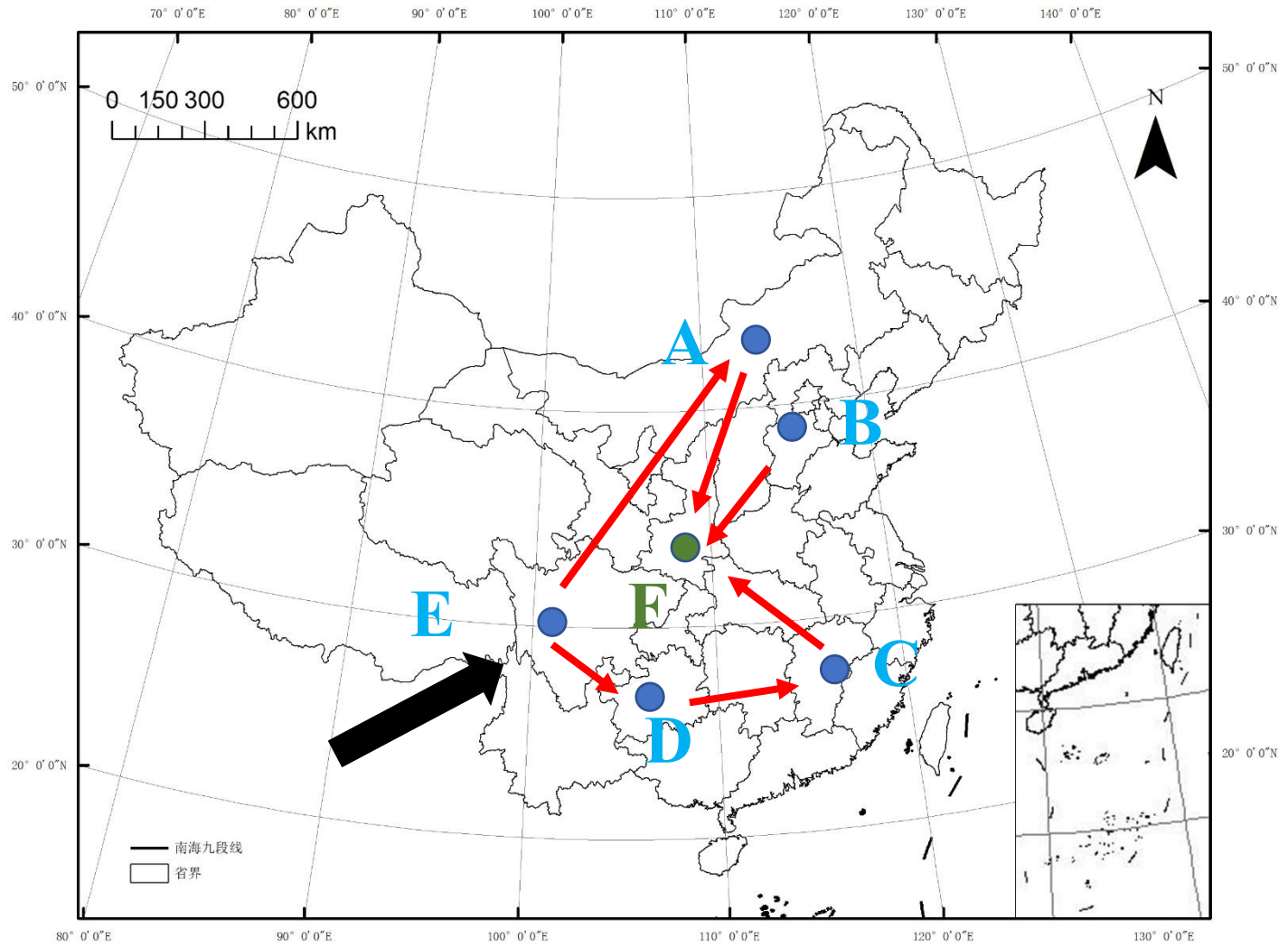




**Figure 1. Satellite map of the reduction of forest area in Rhode Island caused by spongy moth**

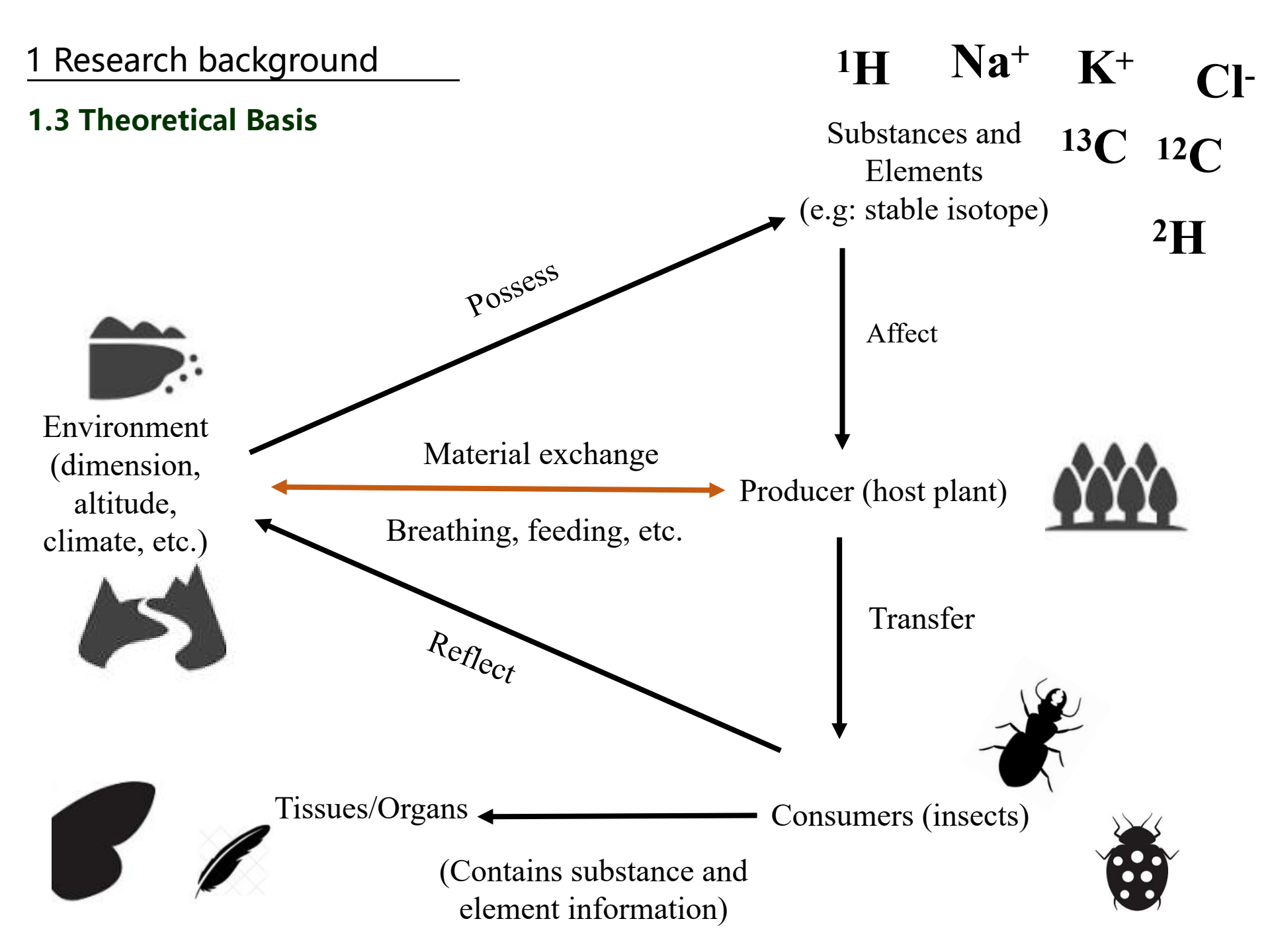
# 1 Research background

## 1.2 Application circumstances

- **Established?**
- **New arrival?**
- **Traceability scale?**



## 1.3 Theoretical Basis



## 1.4 experimental considerations

- The isotope ratio of spongy moth is considered as a regionalized variable reflecting the spatial distribution.
- Establishing an authentic isotope reference database by sampling and detecting spongy moth in different environments.

**Part 1** Hydrogen stable isotope analysis

- Determination of isotope ratio of samples
- Obtain environment variable data set
- Analyze the correlation between precipitation and isotope ratios
- Visual reference mark database

**Part 2** Carbon stable isotope analysis

- Determination of isotope ratio of samples
- Obtain environment variable data set
- Constructing kriging interpolation model
- Visual reference mark database

# 2 Research steps-Hydrogen

## 2.1 Sampling sites

From May 2016 to June 2019, a total number of 284 *L. dispar* adults were captured in five geographic regions

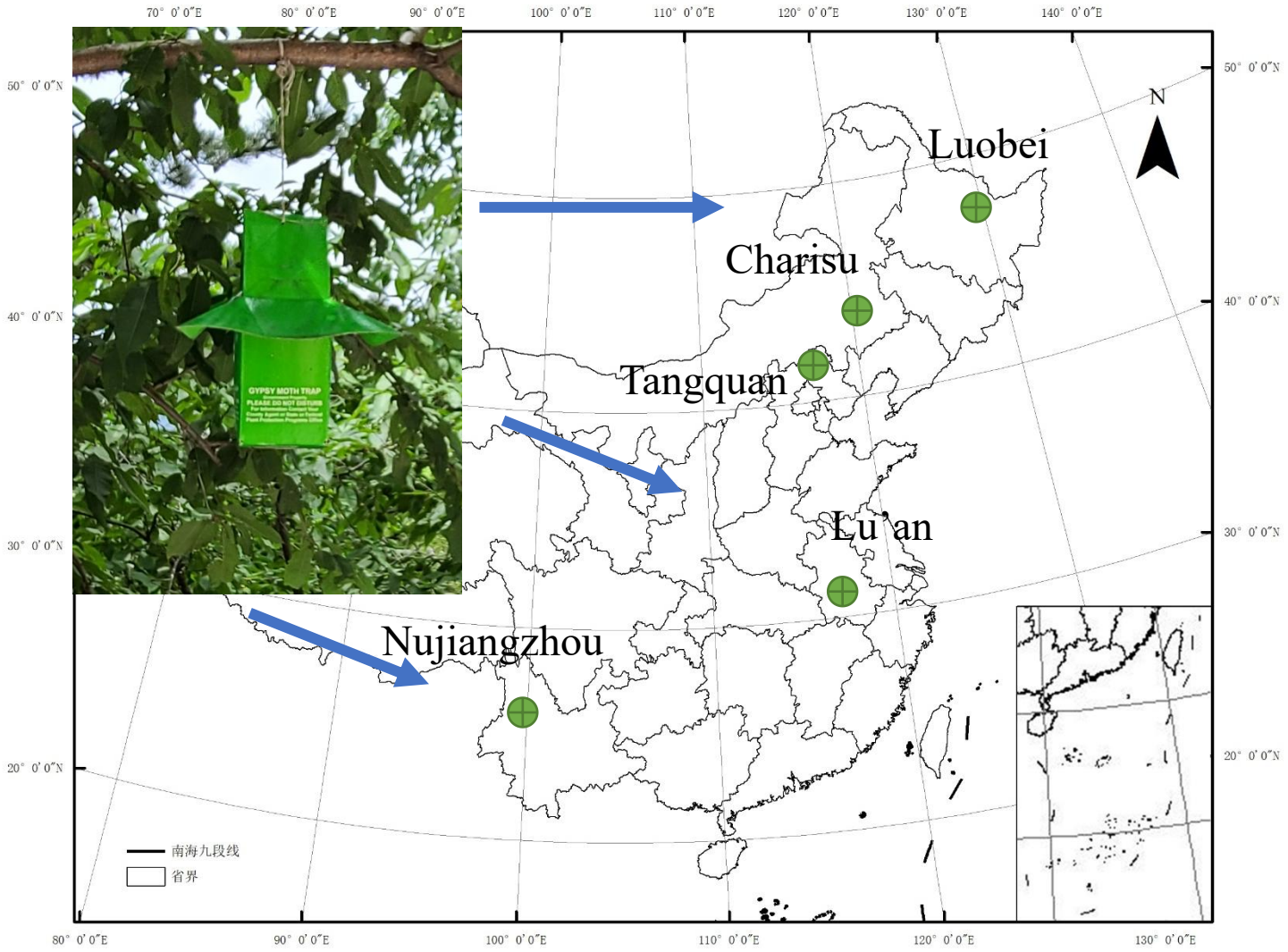
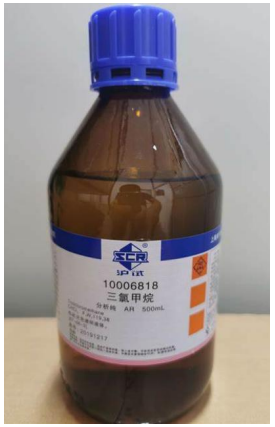



**Figure 2. Sampling location**

# 2 Research steps-Hydrogen

## 2.2 Treatment of research objects

before degreasing



chloroform: methanol

after degreasing

GC-MS

# 2 Research steps-Hydrogen

## 2.3 Environment variable

The grid data of hydrogen stable isotope in global precipitation were downloaded from the Online Isotopes in Precipitation Calculator (OIPC; Bowen and Revenaugh, 2003, Bowen, 2017)

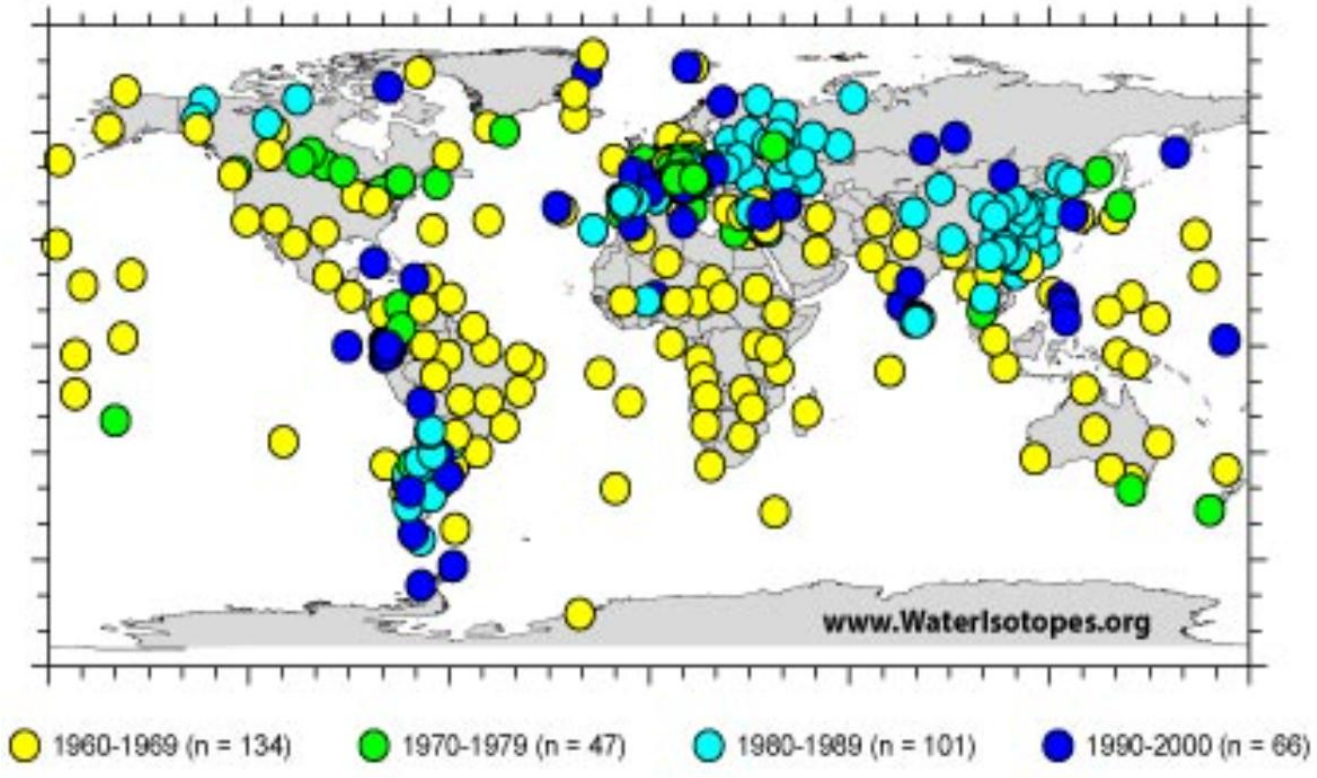



**Fig. 3 Year of $\delta^2H$ Observation at 348 GNIP Stations**

# 3 Research results-Hydrogen

## 3.1 Distribution of $\delta^2H$ Value in Precipitation

$\delta^2H$ value in precipitation had regular changes, with a slight decreasing gradient from southeast to northwest. This isotope base map was later used as the basis for spongy moth source determination.

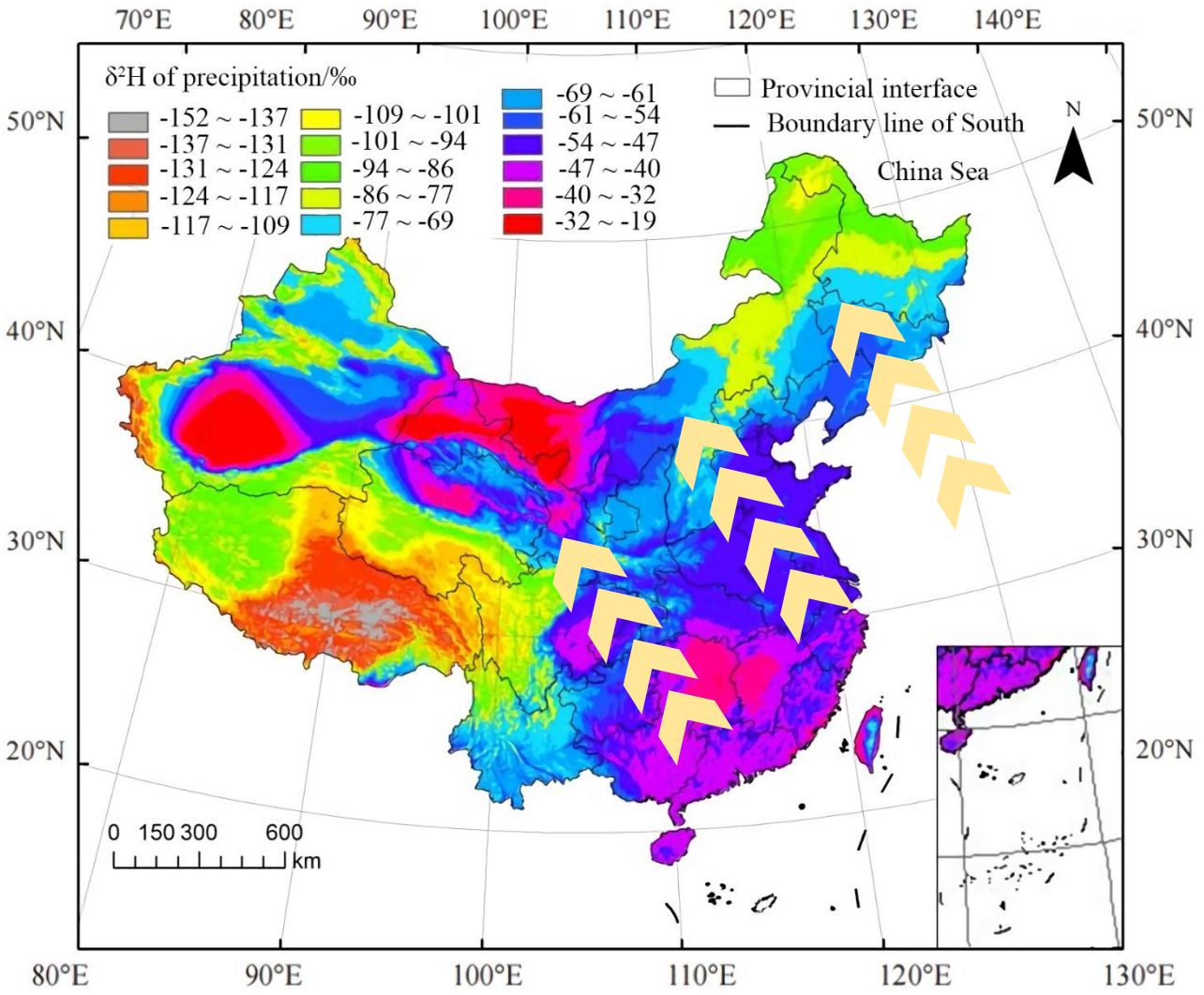



**Fig. 4 Zoning map of hydrogen stable isotopes in precipitation**

# 3 Research results-Hydrogen

## 3.3 Use of Software R and Origin to Model and Test

**Tab. 1 $\delta^2$H value of spongy moth in the five locations in China. Measured data are mean ± sD. $n$ = 3 biological replicates.**

| Place (Regions) | Longitude (East) | Latitude (North) | Height (m) | $\delta^2$H (‰) of *L. dispar* (Measured Value) | $\delta^2$H (‰) of Precipitation (Theoretical Value) |
|---|---|---|---|---|---|
| Heilongjiang Province (Luobei, $n$ = 3) | 130.83 | 47.58 | 83 | −109.4 ± 0.70 | −82 |
| Yunnan Province (Nujiangzhou, $n$ = 3) | 98.77 | 25.86 | 2057 | −96.0 ± 1.75 | −69 |
| Neimenggu Province (Charisu, $n$ = 3) | 123.47 | 43.13 | 121 | −92.9 ± 0.93 | −67 |
| Hebei Province (Tangquan, $n$ = 3) | 117.96 | 40.95 | 135 | −80.4 ± 1.87 | −58 |
| Anhui Province (Lu'an, $n$ = 3) | 116.52 | 31.73 | 75 | −61.0 ± 0.72 | −41 |

**Tab.2 Test results of equation fitting.**

| Items | Value |
|---|---|
| Residual standard error | 1.645 on 13 degrees of freedom |
| Multiple $R$-squared | 0.9913 |
| Adjusted $R$-squared | 0.9906 |
| $F$-statistic | 1474 on 1 and 13 DF |
| $p$-value | $9.09 \times 10^{-15}$ |

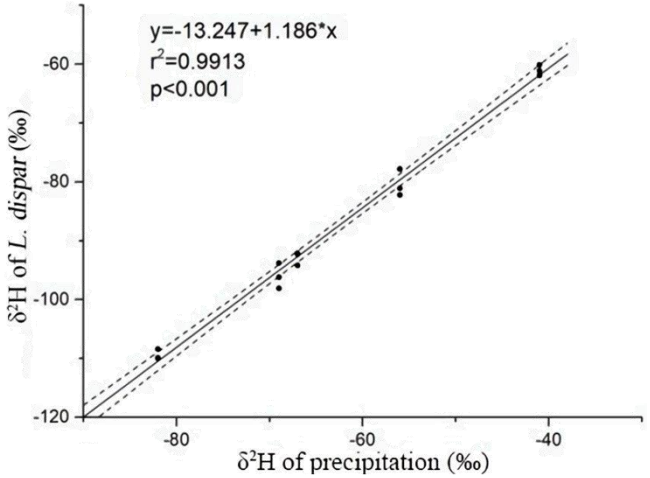



**Fig.5 Standard curve equation.**

# 3 Research results-Hydrogen

y=-13.247+1.186*x

**Tab. 3 Model validation**

| Place (Regions) | Longitude (East) | Latitude (North) | Height (m) | $\delta^2H$ (‰) of *L. dispar* (Measured Value) | $\delta^2H$ (‰) of Theoretical Place (Estimated Value) |
|---|---|---|---|---|---|
| Chengdu Sichuan Province | 104.05 | 30.57 | 488 | −65.2<br>−64.3 | −43.80<br>−43.04 |

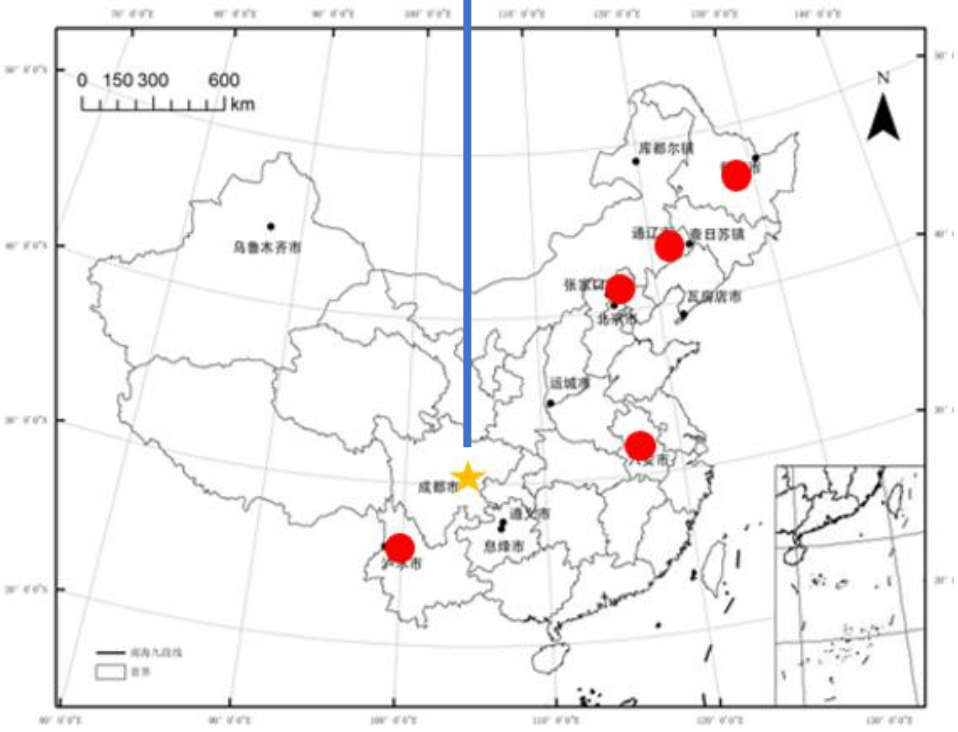



Fig. 6 Schematic diagram of model testing site selection

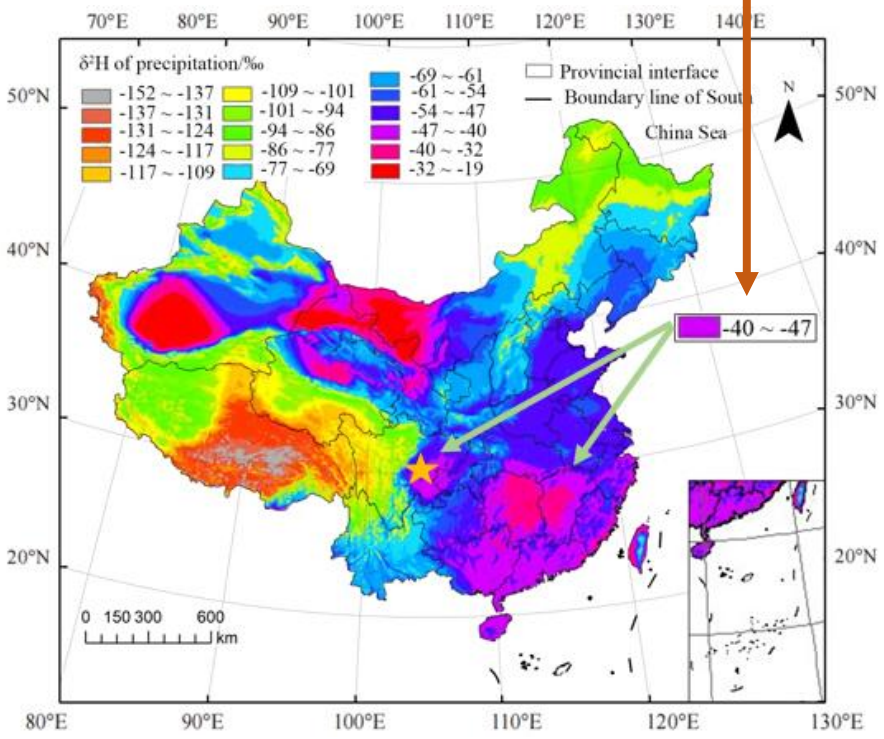



Fig. 7 Schematic diagram of test result indication

# 4 Research steps-Carbon

## 4.1 Sampling sites

In 2020, 423 samples were collected from Yingxian in Shanxi Province, Chunhua in Shaanxi Province, Ulanhot and Kudu in Inner Mongolia, Chengdu in Sichuan Province, and Xifeng in Guizhou Province.

In 2021, 372 samples were collected from Qingdao in Shandong Province, Lianyungang in Jiangsu Province, Panshan in Tianjin, Chifeng in Inner Mongolia, Dalian in Liaoning Province, and Zhaodong in Heilongjiang Province.

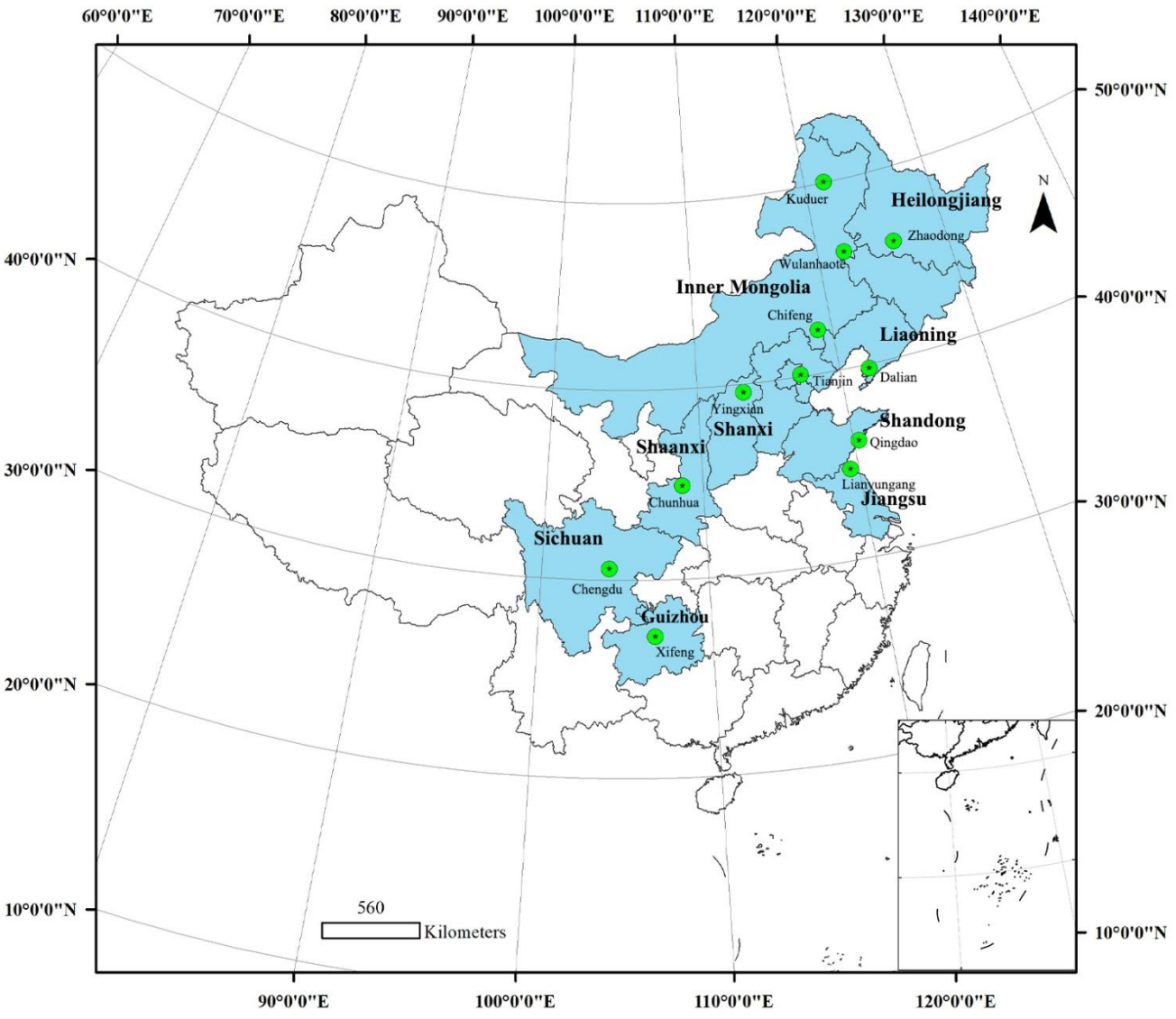



**Fig. 8 Sampling location**

# 4 Research steps-Carbon

## 4.2 Experimental considerations

（1）Filter environment variables

$$\rho_{xy}=\frac{Cov(X,Y)}{\sigma_X\sigma_Y}$$

$Cov(X,Y)$ represents the covariance between any factor X and Y, $\sigma_X$ is the standard deviation of X， $\sigma_Y$is the standard deviation of Y.

The environmental variables screened in the above steps are tested for multicollinearity through the variance expansion factor VIF.

$$VIF_k=\frac{1}{1-R_k^2}$$

It is generally believed that VIF is not greater than 10, and there is no collinearity between independent variables.

**Tab. 4 Environmental Variables**

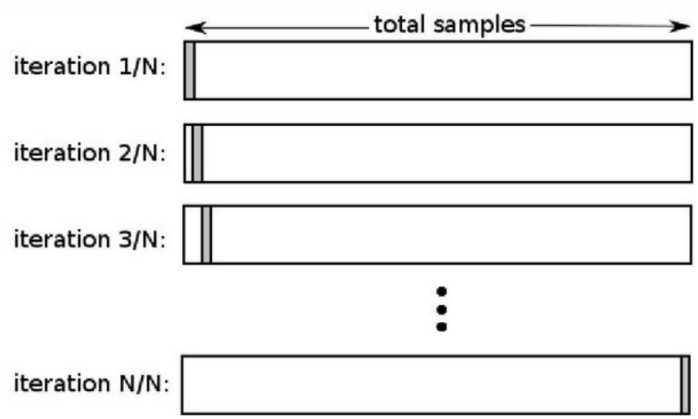
| Site | Longitude | Latitude | Altitude | $\delta^{13}C$ | $BIO_1$ | $BIO_2$ | $BIO_3$ | $BIO_4$ | $BIO_5$ | $BIO_6$ | $BIO_7$ | $BIO_8$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| XF | 106.74 | 27.08 | 750 | -24.63 ± 0.3863 | 14.1 | 7.4 | 28.6 | 700.5 | 27.4 | 0.3 | 27.1 | 21 |
| YX | 113.19 | 39.56 | 1800 | -25.88 ± 0.0528 | 12.5 | 10.8 | 30.1 | 1004.4 | 28.2 | -6.2 | 38.3 | 19.3 |
| LYG | 119.47 | 34.72 | 38 | -27.89 ± 0.0603 | 14 | 8.5 | 25.8 | 926.1 | 29.5 | -2.9 | 32.6 | 25.4 |
| QD | 120.37 | 36.12 | 68 | -26.92 ± 0.0683 | 12.3 | 10.3 | 26.9 | 976.6 | 29 | -6.8 | 35.7 | 24.1 |
| PS | 117.26 | 40.09 | 532 | -25.19 ± 0.1021 | 12.7 | 9.3 | 26.4 | 1103.6 | 30.8 | -9.2 | 39.2 | 25.9 |
| DL | 122.03 | 39.76 | 308 | -27.02 ± 0.1509 | 10.3 | 9 | 23.6 | 1146.8 | 27 | -13.9 | 39.9 | 20.6 |
| ZD | 125.97 | 46.07 | 121 | -29.88 ± 0.0815 | 4.2 | 11.7 | 22.5 | 1532.4 | 27.5 | -24.9 | 52.5 | 21.6 |
| CD | 104.05 | 30.57 | 488 | -26.83 ± 0.1443 | 16.7 | 10.4 | 27.6 | 717.6 | 29.7 | 2.4 | 27.2 | 24.3 |
| KDE | 121.62 | 50.03 | 1058 | -25.17 ± 0.0798 | -4.3 | 14.9 | 26.8 | 1547.9 | 22.1 | -33.6 | 55.8 | 13.9 |
| CF | 119 | 42.3 | 714 | -27.24 ± 0.1756 | 7.5 | 13.2 | 28.7 | 1262.9 | 28.6 | -17.3 | 45.9 | 22.2 |
| CH | 108.67 | 34.85 | 1313 | -26.01 ± 0.0979 | 11.0 | 14.2 | 29.3 | 937.9 | 27.8 | -8.5 | 35.5 | 20.5 |
| WLHT | 122.05 | 46.15 | 264 | -26.18 ± 0.1196 | 3.9 | 13.0 | 28.7 | 1365.1 | 26.9 | -21.5 | 48.5 | 20.6 |

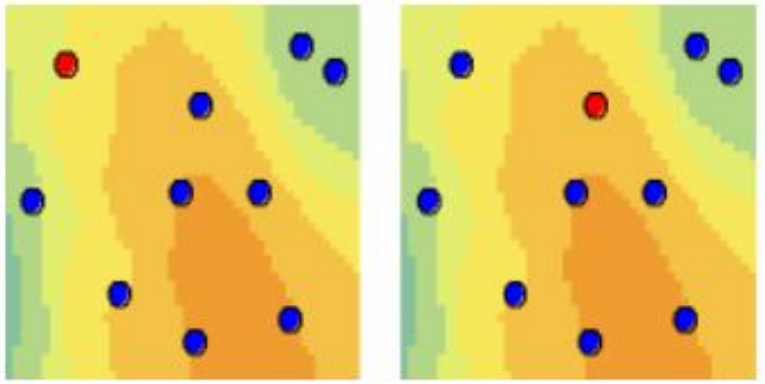
（2） Calculating the correlation between environmental variables and C stable isotope ratio of spongy moth

# 4 Research steps-Carbon

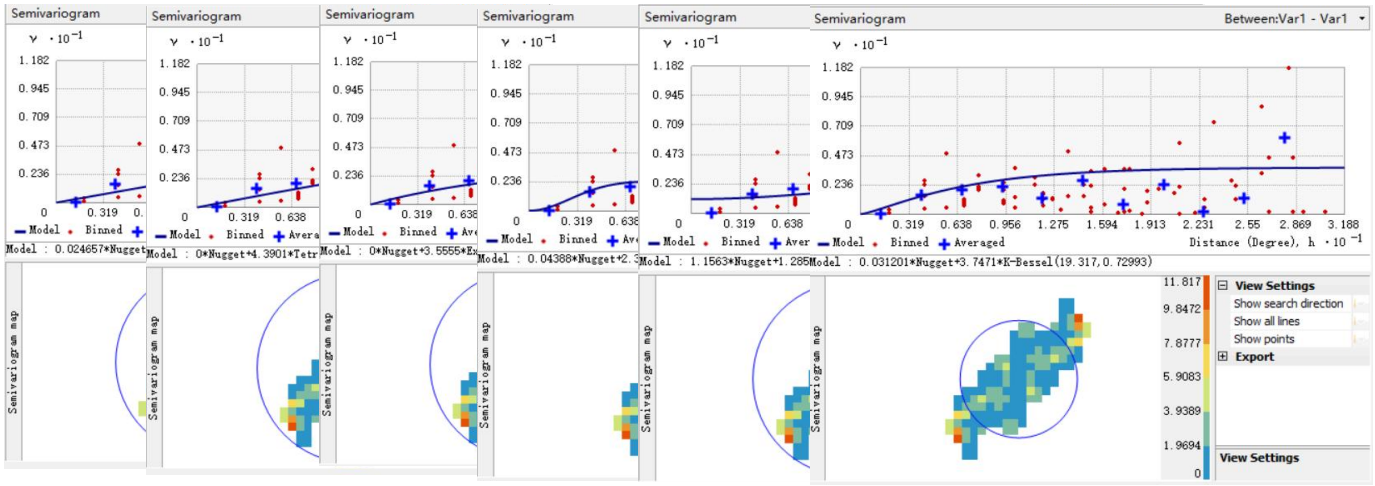
（3） Model validation and accuracy evaluation

"leave one out”



（4） Systematically select candidate models

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Root Mean Square Error

Average Standard Error

Root Mean Square Standardized Error

Mean Standardized

# 5 Research results-Carbon

**Tab. 7 Cross validation（CoKriging with Isothermality）**

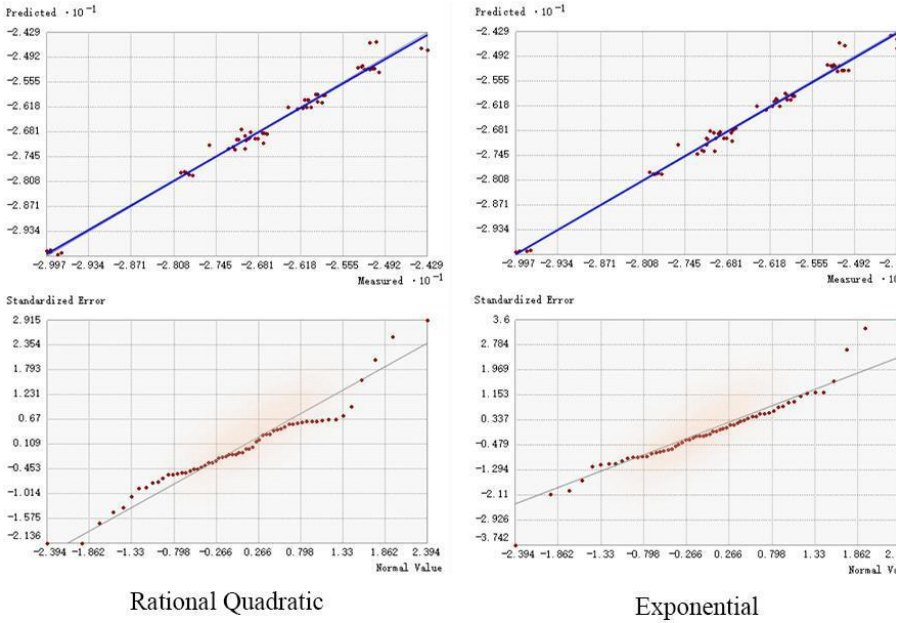
| Model type | Root Mean Square Error | Average Standard Error | Root Mean Square Standardized | Mean Standardized |
|---|---|---|---|---|
| J-Bessel | 0.3001 | 0.2852 | 1.0603 | -0.1374 |
| K-Bessel | 0.1797 | 0.2184 | 0.8259 | -0.0049 |
| Hole Effect | 0.7792 | 1.1348 | 0.6860 | -0.0173 |
| Rational Quadratic | 0.1916 | 0.2077 | 0.9217 | -0.0082 |
| Gaussian | 0.1878 | 0.2354 | 0.7970 | -0.0026 |
| Exponential | 0.1720 | 0.1539 | 1.1751 | -0.0281 |
| Pentaspherical | 0.1721 | 0.1302 | 1.3905 | -0.0333 |
| Tetraspherical | 0.1722 | 0.1288 | 1.4064 | -0.0337 |
| Spherical | 0.1785 | 0.2120 | 0.8494 | -0.0075 |

## Evaluation Criterion:

- Smaller Root Mean Square Error
- Average Standard Error approach Root Mean Square Error
- Root Mean Square Standardized Error approach 1
- Smaller Mean Standardized

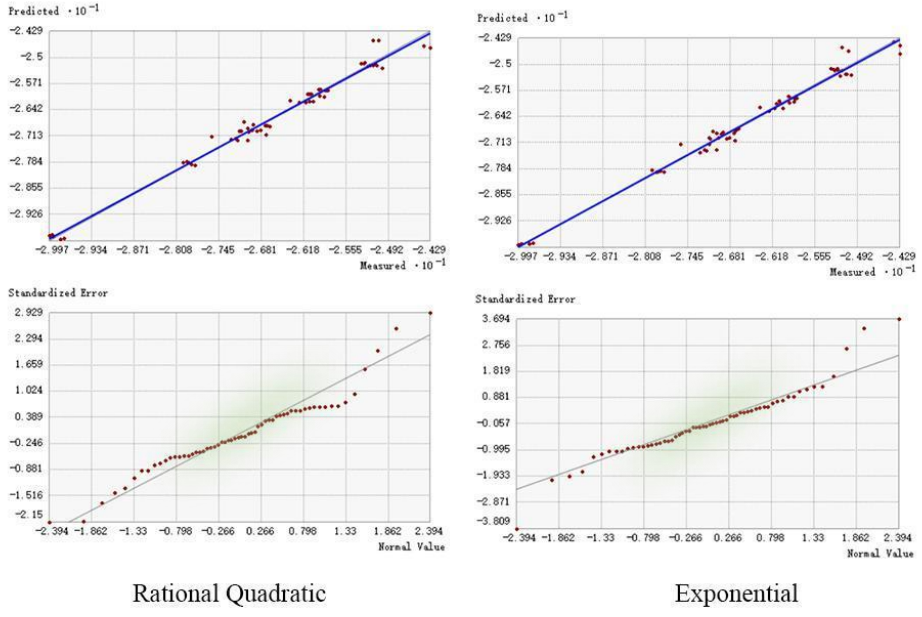
# 5 Research results-Carbon

Exploratory Spatial Data Analysis (ESDA)




Rational Quadratic Exponential

**Fig. 9 CoKriging with Isothermality**




Rational Quadratic Exponential

**Fig. 10 CoKriging with Altitude**

# 5 Research results-Carbon

- The SD of C isotope ratio of samples in the same area is ± 0.3863 (n=5).
- The maximum difference of C isotope value among the same location is 0.7726

- When the number of classification groups is 5, the interval difference of C isotope value is about 1.00 (0.7726 < 1.00)

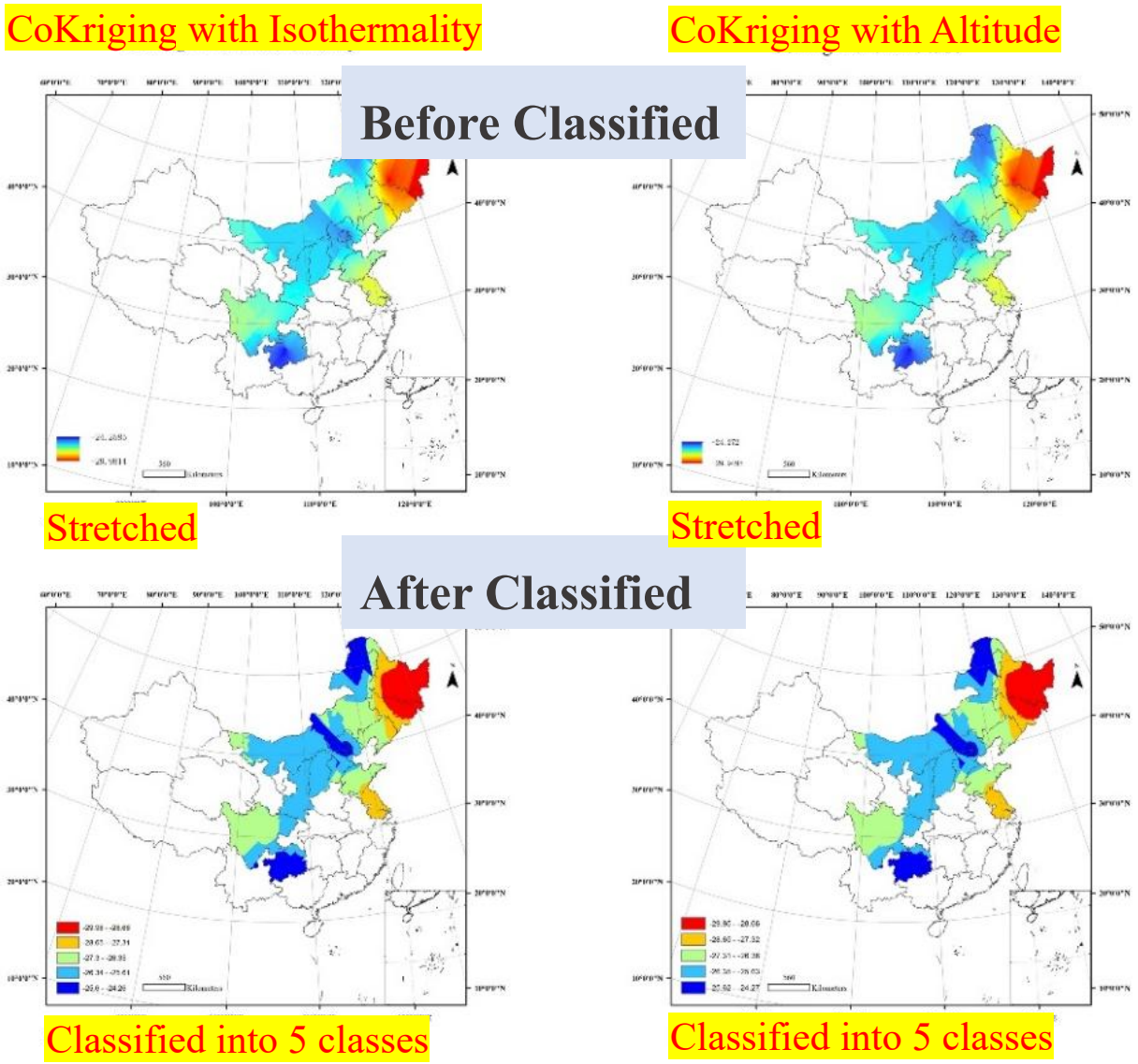



**Fig. 11 Visualized indication model**

# 4th Conference organized by IFOPE

2023.1.18

Thank you for
your attention

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